Studies on Pyrazolo (3, 4-d) pyrimidine Nucleosides

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BY

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled "Studies on Pyrazolo[3,4-d]pyrimidine nucleosides" has been carried out by Mr.Shoeb Iqbal Khan, under our supervision.

He has fulfilled the requirements of the Aligarh Muslim University regarding the prescribed period of investigational work for the award of M.Phil. degree.

The work included in this thesis is original unless stated otherwise, and has not been submitted for any other degree.

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dedicated to my loving Mother and Father

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(SHOEB IQBAL KHAN)

CONTENTS

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PREFACE

Nucleosides, the nitrogen glycosides of purines and pyrimidines and their phosphate esters, known as nucleotides are vital components of all living cells and are intimately involved in many biological processes, such as protein synthesis, storage and transformation of genetic information, oxidation, reduction and electron transport processes.

Living cells contain a variety of low molecular weight nucleotides, which have important roles as reaction intermediates in the complex integrated chemical reactions of the cell. They synthesize their requirement of purine and pyrimidine nucleotides from amino acids.

Modification of heterocyclic and/or sugar moieties of purine nucleosides affords modified nucleosides which may either inhibit or act as a competitive substrate of some important enzymes and thus exhibit a variety of biological activities.

In the thesis the synthetic analogues of biologically active nucleosides and their biological evaluation is described. It is divided into two chapters.

The first chapter is a general review and deals

with acyclic nucleosides of biological interest.

The second chapter deals with the synthesis of acyclic nucleosides of 4-substituted pyrazolo[3,4-d]pyrimidine and their biological activity.

ABBREVIATIONS

ATP	: Adenosine triphosphate
AMP	: Adenosine monophosphate
IMP	: Inosine monophosphate
RNA	: Ribonucleic acid
DNA	: Deoxyribonucleic acid
HSV	: Herpes simplex virus
GMP	: Guanosine monophosphate
PRPP	: Phospho ribosyl py rophosphte
Ara-A	: Adenosine arabinoside

CHAPTER - I

Acyclic Nucleosides of Biological Interest

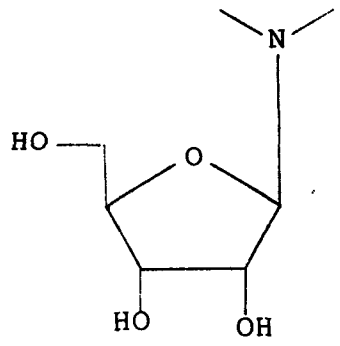
1.1 INTRODUCTION

The nitrogen heterocyclic molecules are of considerable interest to chemists, biochemists and medicinal chemists and provide interesting problems for synthesis and to study the mechanism of biological processes at molecular level. They also provide a 'Lead compound' for designing new drugs for a variety of human ailments. Several nitrogen heterocycles are elaborated by both animal and plant cells. Of these purine and pyrimidine nucleosides and nucleotides are of considerable interest.

The term "nucleoside" introduced by Levene and Jacobs[1] in 1909, was originally used for carbohydrate derivatives of purine and/or pyrimidine, isolated from alkaline hydrolysates of yeast ribonucleic acid (RNA) and enzymetic digestion of thymus deoxyribonucleic acid (DNA)[2]. However, the current definition of nucleosides is very broad. It includes all those compounds in which a nitrogen heterocyclic moiety is linked to a glycon moiety. The heterocyclic moiety may be a substituted purine, modified purine such as pyrazolo pyrimidine, pyrrolo-pyrimidine, aza and deaza purines, substituted pyrmidine and a modified pyrimidine base. The glycon moiety may be ribose, deoxyribose or any other sugar,it can also be an alcohol and amino alcohols moiety.

1.2 TYPES OF NUCLEOSIDES

Nucleosides can be broadly classified into N-nucleosides (1) and C-nucleosides (2). In N-nucleosides, the anomeric carbon atom of sugar is attached to heterocyclic moiety by C-N bond, whereas in C-nucleoside, the anomeric carbon atom of sugar is attached to heterocyclic moiety by C-C single bond.



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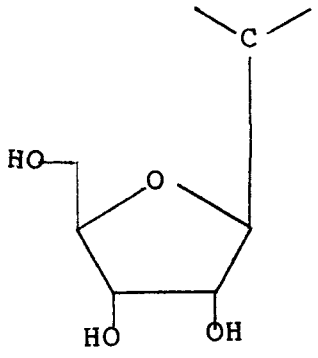

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The compound in which the glycone moiety has a cyclic structure like ribose, xylose, arabinose etc. are called 'cyclic nucleosides' and the compounds in which an open chain structure constituted the glycone moiety, called 'acyclo nucleosides'.

1.3 BIOLOGICAL IMPORTANCE OF NUCLEOSIDES

Nucleosides and nucleotides have attracted considerable attention, not only because they are building blocks of nucleic acid DNA and RNA, but also they are co-factors and allosteric effectors in many of the

fundamental enzymic reactions.

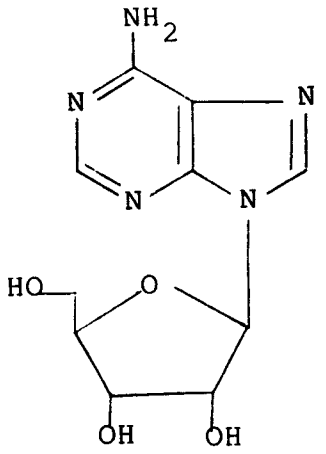
Excellent reviews dealing with various aspects of purine and pyrimidine nucleosides are available in literature[3-12]. Analogues of natural purine and pyrimidine nucleosides have proved to be quite effective as antibacterial, antiviral, anticancer and antitumor agents, due to their role as enzymes inhibitors and agonist/antagonists.

Adenosine (3), deoxyadenosine (4), guanosine (5), deoxyguanosine (6), cytidine (7), deoxycytidine (8), uridine (9) and thymidine (10) are the component of nucleic acid are given in Fig. 1.

1.4 NATURALLY OCCURING MODIFIED NUCLEOSIDES

A variety of modified nucleosides have been isolated from plants and mammalian DNA. A numer of interesting nucleosides that have been isolated from marine organisms are spongouridine (11), doridosine (12), 3'-acetyl spongosine (13) and spongosine (14) are given in Fig. 2.

Another group of modified purine and pyrimidine nucleosides that have been isolated from microorganism, and exhibited antibiotic activity are called as "nucleoside antibiotics". Some of the nucleoside antibiotics in which the heterocyclic moiety is modified are formycin (15), nebularin (16), cadeguamycin (17), toyocamycin (18), bredenin (19), showdomycin (20) and pyrazomycin



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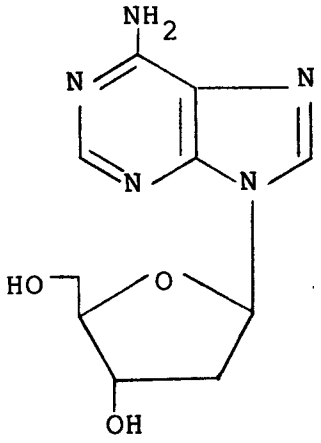

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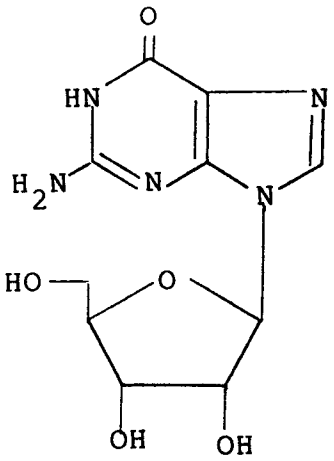

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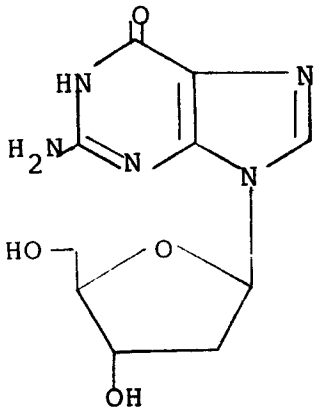

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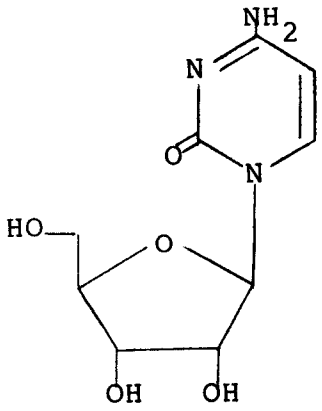

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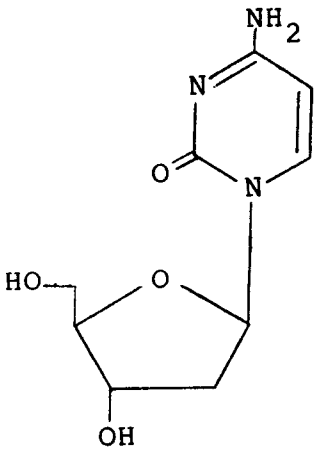

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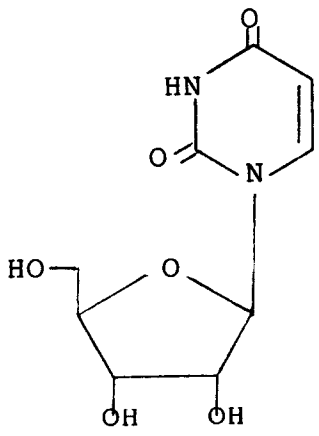

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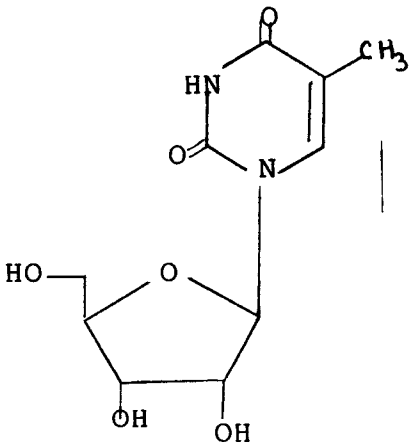

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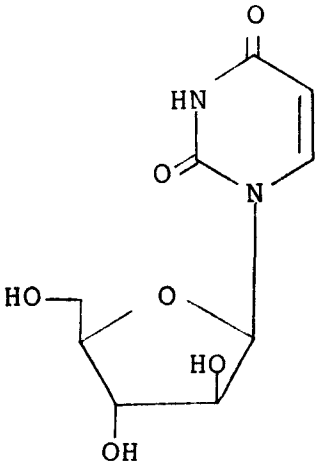
Fig.1



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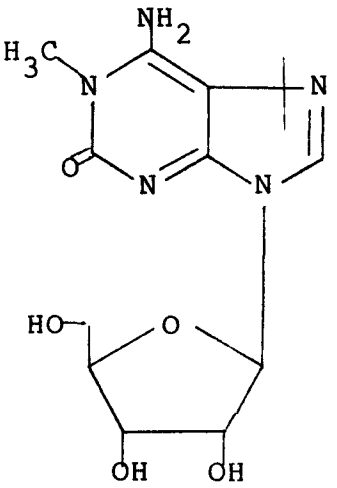

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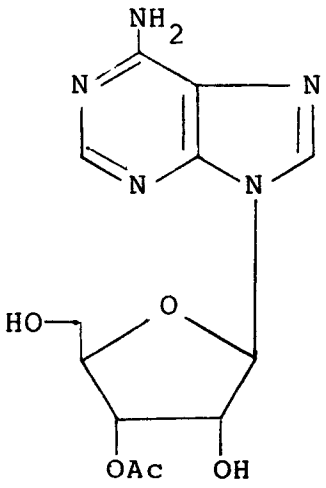

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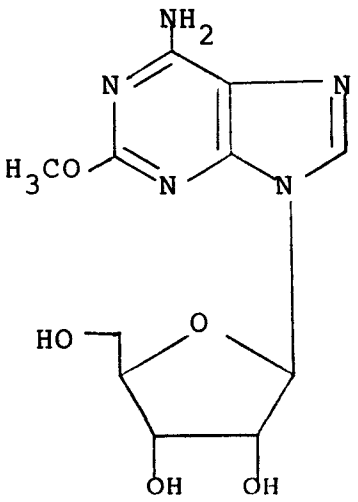

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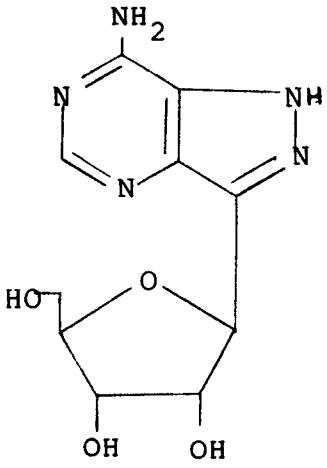
Fig.2



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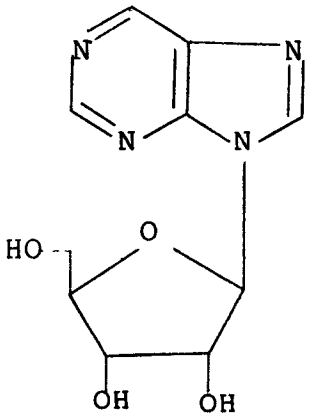

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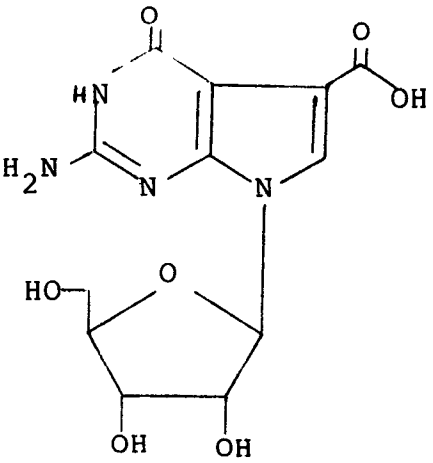

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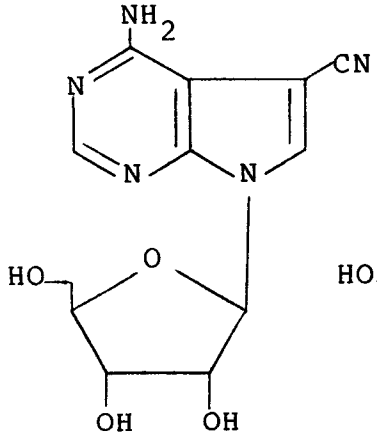

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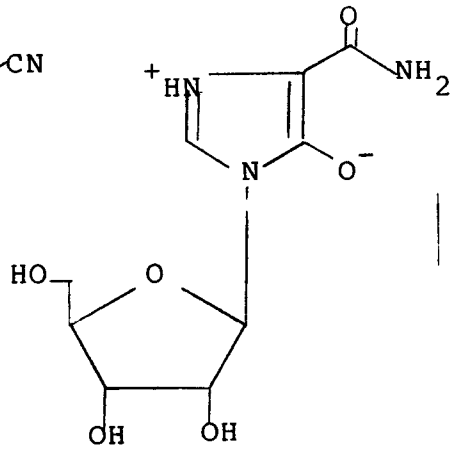

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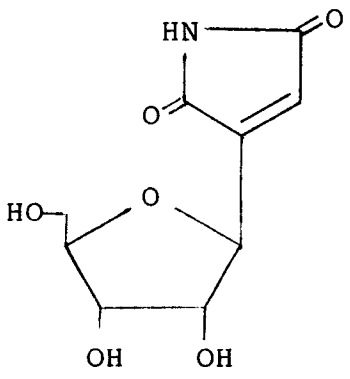

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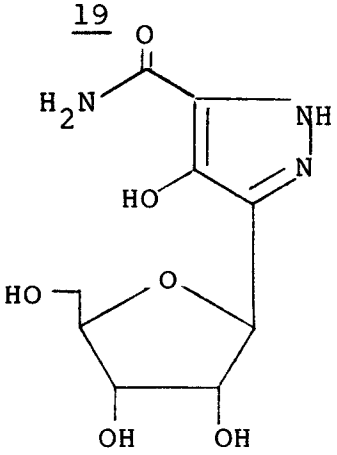

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Fig.3

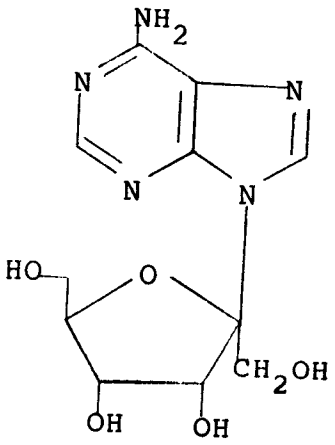
(21) are given in Fig3. The nucleoside antibiotics in which the glycon moiety is modified are psicofuramycin (22), cordycepin (3'-deoxyadenosine) (23), 3'-amino-3'-deoxyadenosine (24), 3'-acetamido-3'-deoxyadenosine (25), homocitrullyl amino adenosine (26), lysylaminoadenosine (27) and decoyinine (28) are given in Fig. 4.

There are several nucleoside antibiotics in when both the heterocyclic and glycon moieties are modified. Example of these class of nucleoside are puromycin (29), 5'-deoxy-5'-iodotubercidin (30), amicetin (31) and plicacetin (32) are given in Fig. 5.

1.5 SYNTHETIC MODIFIED NUCLEOSIDES

The biological activity of a nucleoside can be modulated by making chemical modification in heterocyclic and/or carbohydrate moiety or by changing the configuration of the hydroxyl function in sugar moiety.

In recent years, several nucleosides have been obtained by replacing the normal sugar moieties such as D-ribose and D-deoxyribose by a variety of non-conventional acyclic alcohols. The compounds of this type are termed as 'acyclic nucleosides', which exhibit high order of biological activities. Depending on the substituent in the heterocyclic moiety, acyclic nucleoside could be divided into two groups, the guanine and the adenine.



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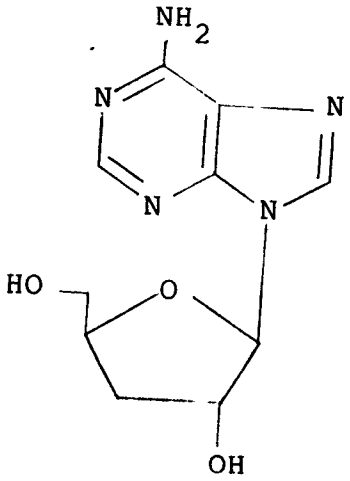

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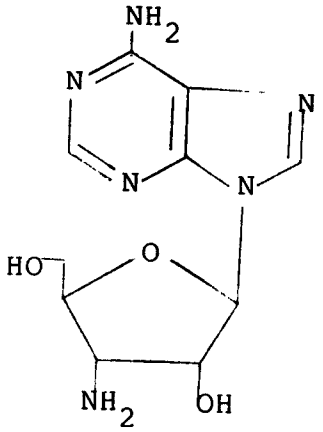

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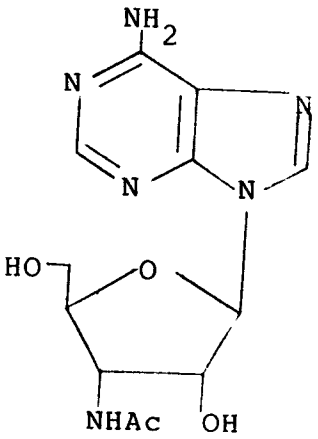

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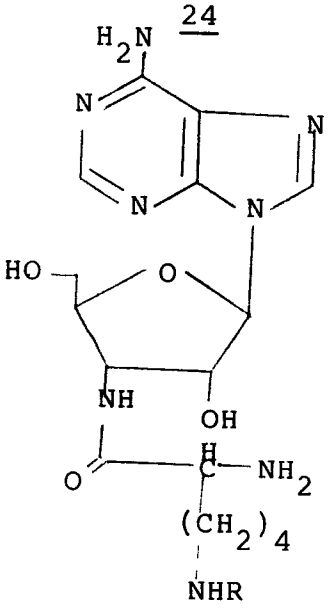

26, R= $OCNH_2$

27, R=H

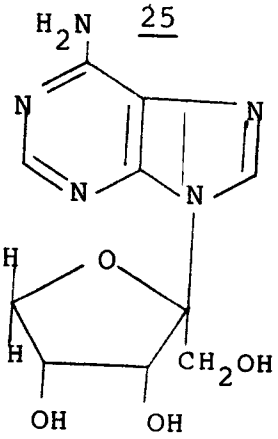

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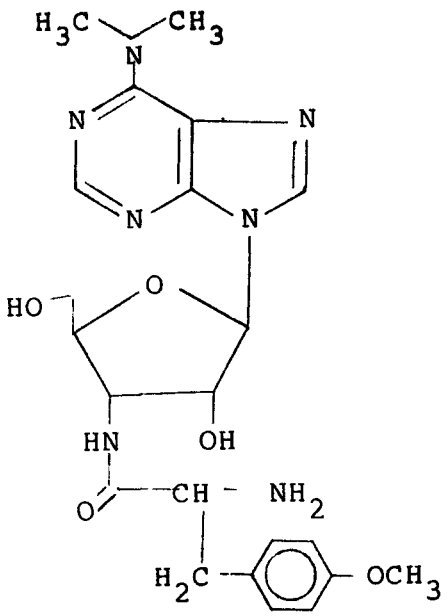
Fig.4



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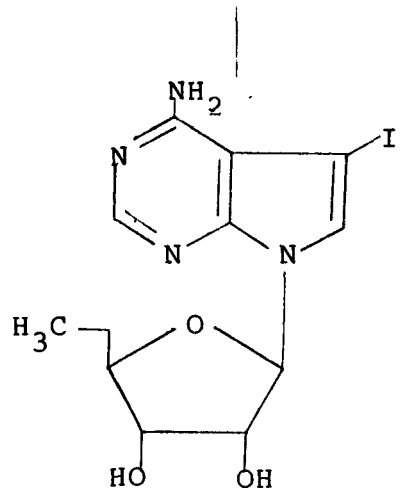

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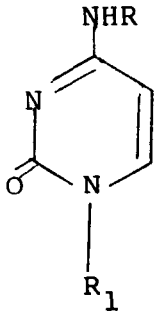

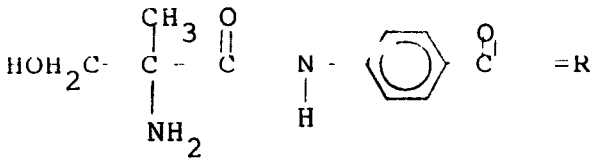

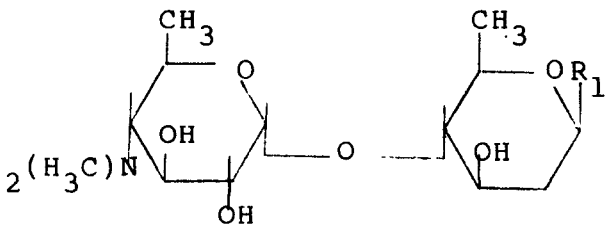

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Fig.5

1.5.1 Guanosine Analogues

In the nucleosides of this class D-ribose moiety of the nucleoside is replaced by 2-hydroxy ethoxy methyl[13], amino acyl esters[14], 1,3-(dihydroxy-2-propoxy)methyl[15-17], 4-hydroxybutyl[18], 3,4-dihydroxybutyl group[19].

1.5.2 Adenosine Analogues

In this class of nucleosides the D-ribose moiety of the nucleoside is replaced by the aliphatic side chain such as 2,3-dihydroxypropyl[20], 2,3-dihydroxybutyl[21] and 2,3-dihydroxybutanoic acid (as in the eritadenine)[22].

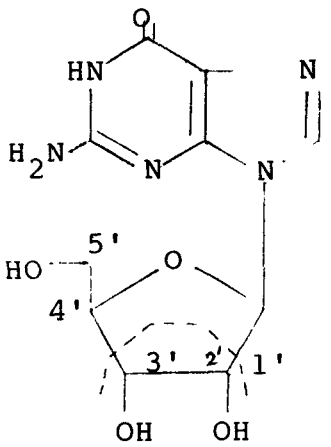
The guanine acyclic nucleosides are specifically active against herpes simplex virus[13-19], cytomegalo virus[23,24] and Epstein Barr virus[25], while the adenine acyclic nucleosides are S-adenosyl-L-homocystein hydrolase inhibitors or adenosin deaminase inhibitors. They show a wide spectrum of antiviral activities against DNA and RNA viruses including poxviridase (vaccinia), rhabdoviridase (rabies, vericular stomatitis), paramyxoviridae (measles, parainfluenza) and reoviridae (reo, rota).

1.6 GUANOSINE NUCLEOSIDES ANALOGUES

1.6.1 Hydroxy ethoxy methyl guanine (acyclovir) analogue

Significant progress has been made in the development of antiviral chemotherapy[6,26-36]. A number of

nucleosides effective against Herpes virus have been synthesized. Replacement of ribofuranosyl moiety of guanosine with 2-hydroxy ethoxy methyl moiety yielded 9-(2-hydroxyethoxymethyl)guanine (acyclovir)[13] (33), a potent antiviral drug. In acyclovir (33) the base -C(1')-O-C(4')-C(5') fragment of guanosine (5) is present.



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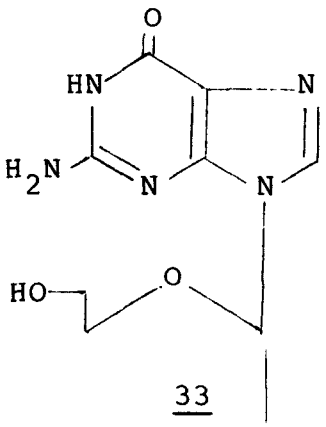
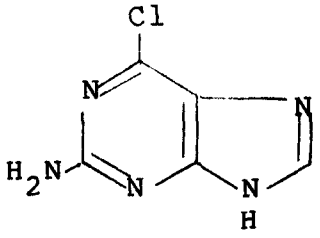


33

Schaeffer et al[13] have reported the synthesis and antiherpes activities of acyclovir. It is a very potent inhibitor of herpes simplex virus type-1 (HSV-1), type-2 (HSV-2), varicella zoster virus (VZV) and cytomegalo virus replication and produces little cytotoxicity in uninfected cells[36,37]. Acyclovir is specifically phosphorylated to its 5'-monophosphate by herpes virus thymidine kinase, but not by cellular thymidine kinase[37,38]. Acyclovir triphosphate, synthesized by cellular kinase from monophosphate is more potent inhibitor of the virus.

Several acyclovir analogues (34-42) have been synthesized and some of them exhibit good antiviral activity.



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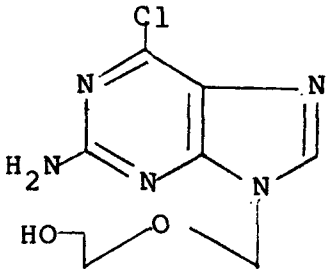

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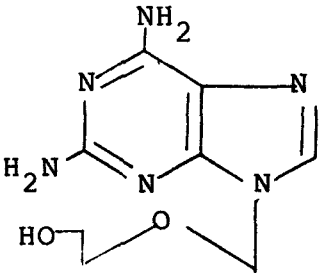

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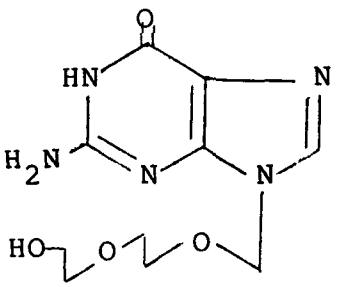

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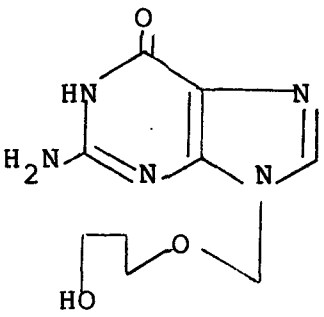

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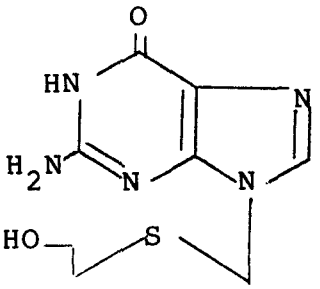

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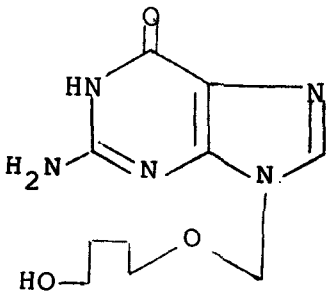

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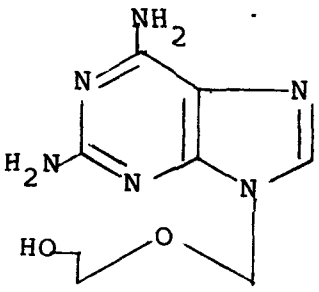

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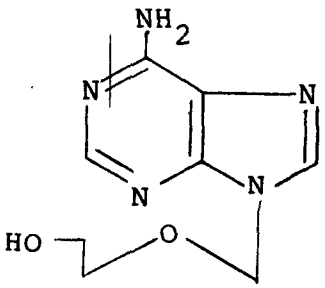
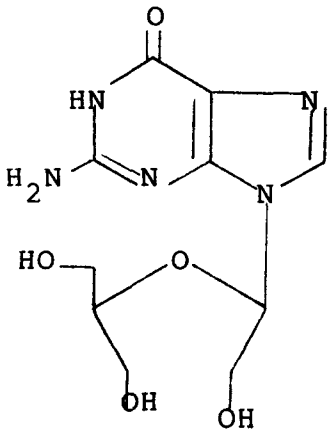


42

Since the compounds with guanine moiety (37-40) were found more potent than with adenine (42) or 2,6-diamino purine moieties (41), several acyclonucleosides with guanine moiety (43-48) were synthesized[39].



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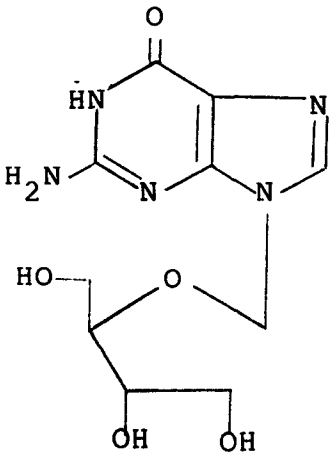

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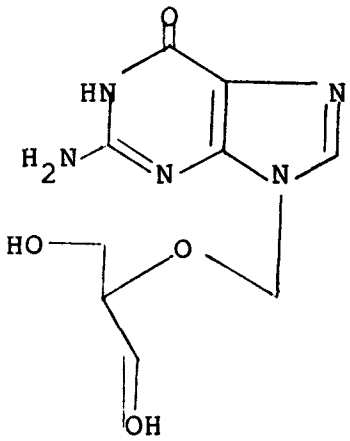

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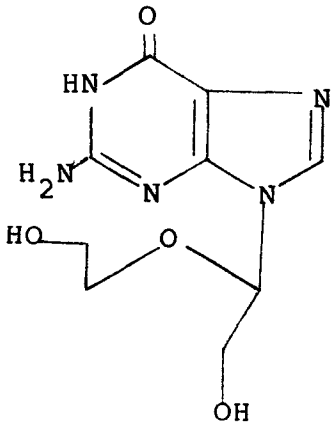

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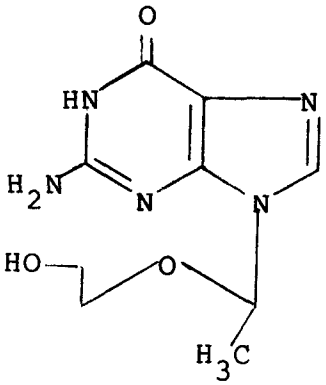

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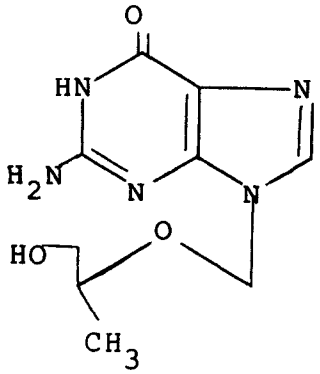
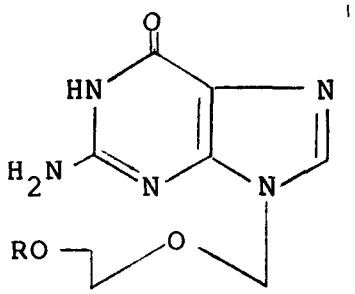


48

Correlations with antiherpetic activity and enzymatic phosphorylation of various acyclovir analogues have been studied by Keller et al[40] and Kelley[41]. It has been found that nucleosides although are phosphorylated but do not exhibit viral activity which suggest that phosphorylation catalysed by the thymidine kinase alone is not sufficient for inhibition of viral replications.

Antiviral efficacy in HSV-1 keratitis in rabbit was found in compound (49).



49, R=$COCH_2NH_2.HCl$

50, $COCH(CH_3)NH_2.HCl$

51 $COCH_2CH_2NH_2.HCl$

52 $COCH_2CH_2COONa$

53 $COCH_2N_3$

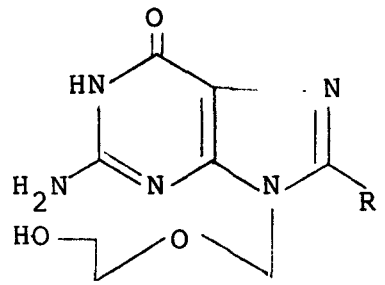
54 $COCH(CH_3)NHCOOCH_2C_6H_5$

55 $COCH_2CH_2NHCOOCH_2C_6H_5$

56 $COCH_2CH_2COOH$

Vanderhaeghe and coworkers[42] synthesized and evaluated the antiviral activity of compounds (49-56).

A number of 8-substituted purine acyclonucleosides were prepared by Robins et al[43] for antiviral, antimetabolic and cytotoxic properties. Among these the 57-60 analogues exhibited significant activities.



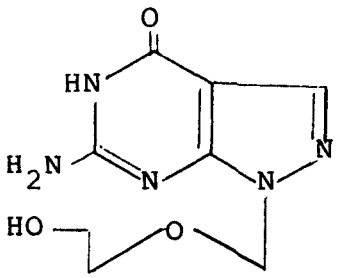
57, R = CH_3

58, Br

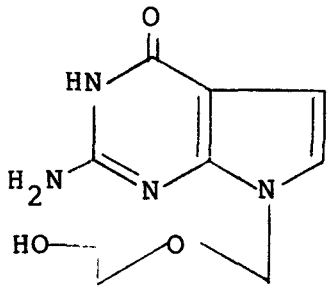
59, I

60, NH_2

Acyclovir analogues (61-64) were reported by Beauchamp et al[44], with various heterocyclic moieties such as pyrazolo[3,4-d]pyrimidine (61), pyrrolo[2,3-d]pyrimidine (62)[45], benzoguanine (63), 8-azapurine (64), imidazole, triazole and isocytosine.



61



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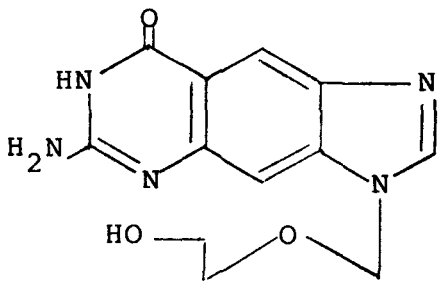

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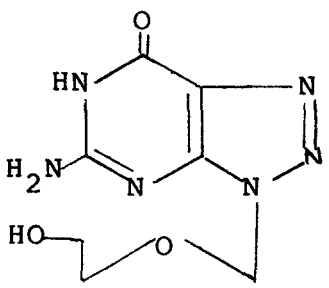


64

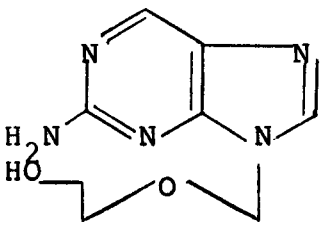
Comparative binding studies of acyclovir and nucleoside (64) with thymidine kinase had been reported.

Synthesis of 6-deoxyacyclovir (65) as prodrug was reported by Kreintsky et al[45]. The deoxyacyclovir was found to be 18 times more water soluble than acyclovr. Furthermore, the 6-deoxyacyclovir was readily oxidised to acyclovir by xanthine oxidase. Another acyclovir derivatives 2,6-diamino-9-(2-hydroxyethoxy)methylpurine (A 134U)[46-48] (41) was studied as prodrug. A 134U (41) was converted into acyclovir by adenosine deaminase and four other minor metabolites[46-48].

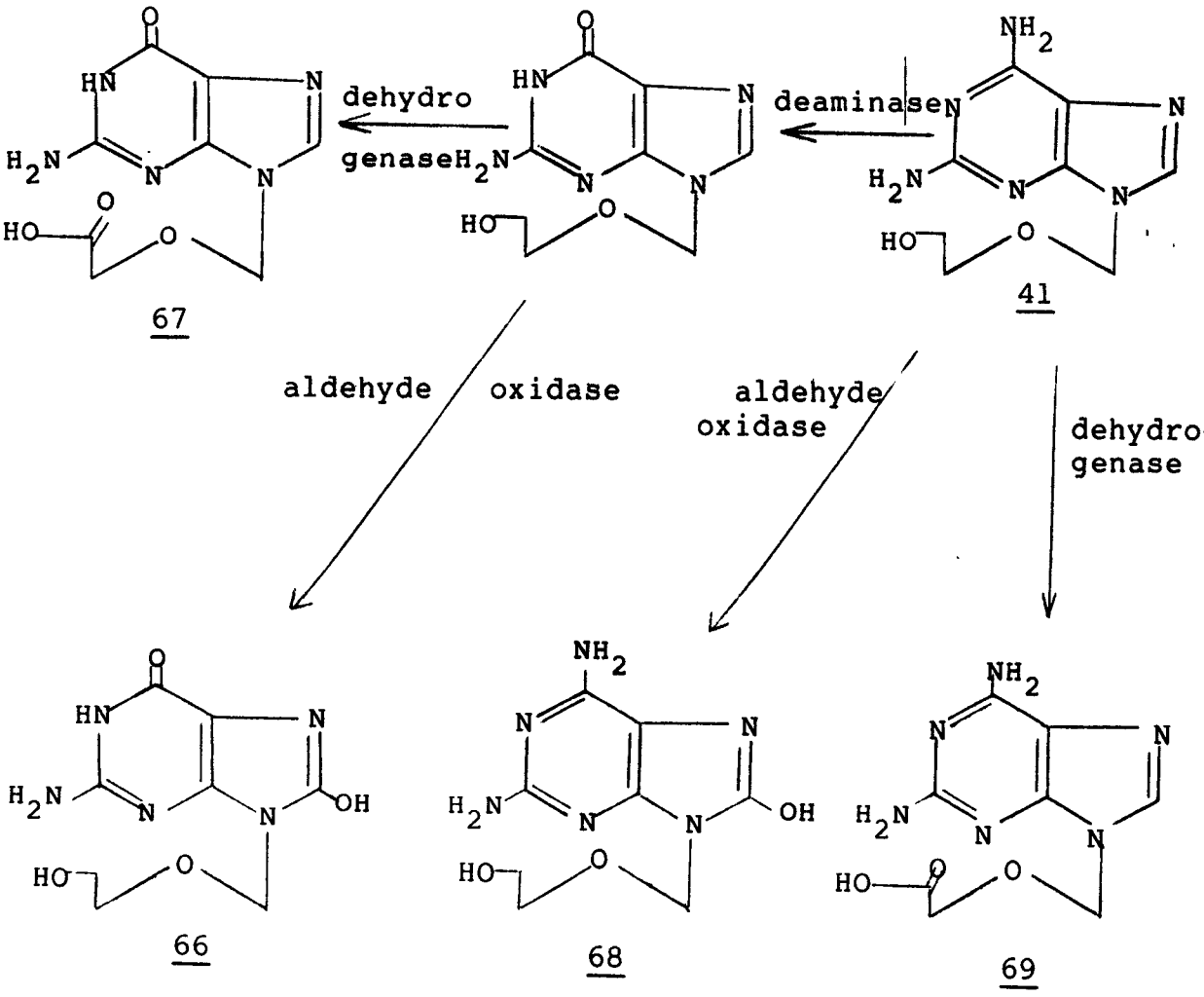
1.6.2 9-(3,4-Dihydroxybutyl)guanine (DHBG)and analogue

9-(3,4-Dihydroxybutyl)guanine (DHBG) (70) showed significant antiperpes activity[49,50]. DHBG was selectively phosphorylated by HSV thymidine kinase. The (R)-enantiomers (70-a) was found to inhibit viral multiplication than the (S)-enanthiomers (70-b). DNA synthesis of herpes virus was selectively inhibited by (RS)-DHBG (70-c) in infected cells. Further the compounds was found to have low cellular toxicity.

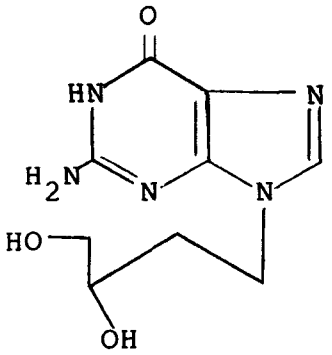
Antiviral activity of DHBG had also been compared with other related acyclonucleosides[51]. DHBG isomer (71)[52] was found to have low order of antiviral activity. L-erythroisomer (72)[53] was synthesized from L-arabinose



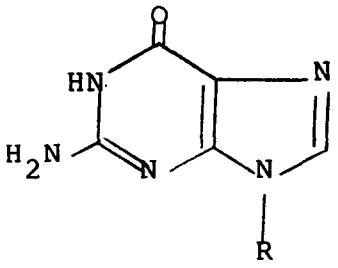
65


dehydro
genase
deaminase
aldehyde oxidase
aldehyde oxidase
dehydro-
genase
67
41
66
68
69

which stereochemically represents the "bottom half" of the D-ribofuranose moiety. However, this compound did not show any activity against herpes and polioviruses.



70

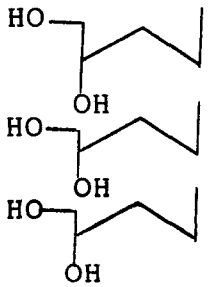


R

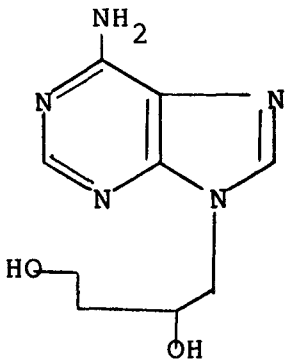
70-a

70-b

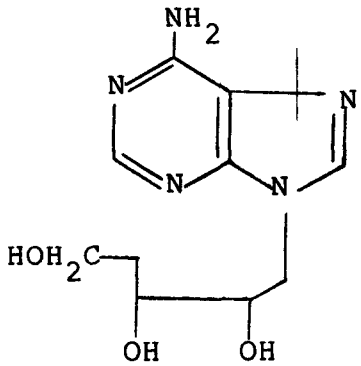
70-c



(S)-DHBG



71



72

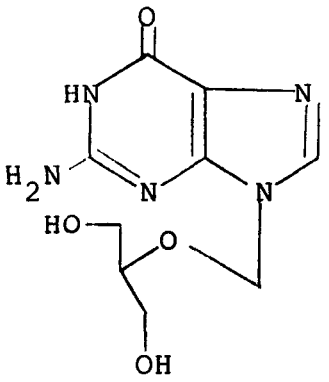
1.6.3 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine (DHPG) analogues

Several analogues of acyclovir had been synthesized. Some of these had shown high order of antiviral activities[54-56]. DHPG (73) is one of such nucleosides, which had been found very promising[56-61].

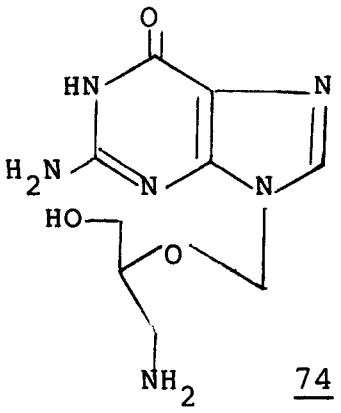
DHPG (73) had similar activity of acyclovir against HSV-1 and HSV-2 *in vitro*. DHPG also inhibited cytomegalovirus and Epstein-Barr virus[56-63]. Although two compounds acyclovir and DHPG had similar activity against HSV *in vitro*. A striking difference was that of DHPG exhibited higher activity *in vivo* against herpes encephalitis and vaginites[56-59]. Mode of action of DHPG and acyclovir were similar. Both the compounds are phosphorylated by viral induced thymidine kinase and both selectively inhibit the virus DNA synthesis[64-66]. Virus induced thymidine kinase first phosphorylated the DHPG to its monophosphate. It is subsequently phosphorylated by host cellular enzymes into its 5'-di and finally to its 5'-triphosphate derivative. Thus in contrast with acyclovir, DHPG seemed to be incorporated into DNA strands[66].

Various analogues of DHPG had been synthesised due to the significant *in vivo* antiviral activities. Lin and coworkers[67] had reported the synthesis of amino analogues (74). The reason for the synthesis of (74)

and its analogues was that the amino group is sterically and electronically similar to that of the hydroxyl group. Which may lead to the compound with high antiviral activities and low host cytotoxicity. Compound (74) exhibited moderate antiviral activity[68].



73



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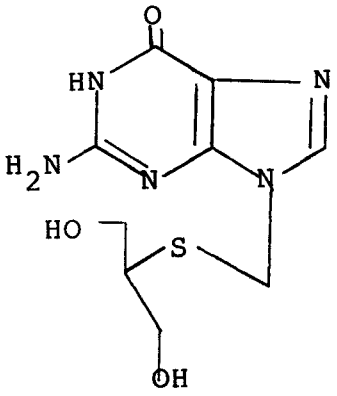
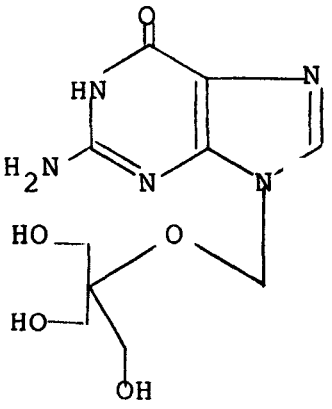


75

Thio analogues (75) of DHPG had also been synthesized by McGee et al[69]. The compound (75) exhibited comparable activity in vitro to DHPG against HSV-1 and human cytomagalovirus.

Ogilvie et al[70] had synthesized the DHPG analogues (76-80) with an additional hydroxy methyl group on the acyclic side chain. This modification decreased the herpetic activity. Further side chain elongation as in (81)[71] had significantly reduced antiviral activity HSV-1, HSV-2 and cytomegalovirus. Another DHPG analogues (RS)-9-(2,3-dihydroxy-1-propoxymethyl)guanine (82) has been reported by both Lin and Lin[72] and Ashton[73]. (R)- and (S)-enantiomers[74] had also been synthesized. The (S)-isomer (83) was found to be 10 to 25-fold more action than (R)-isomer (84) against HSV-1 and HSV-2 in cell culture.



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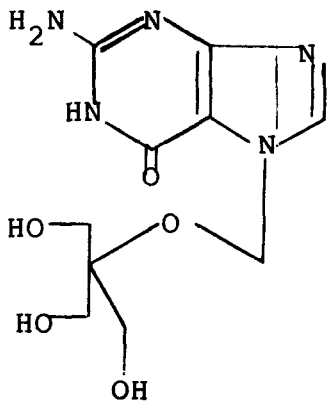

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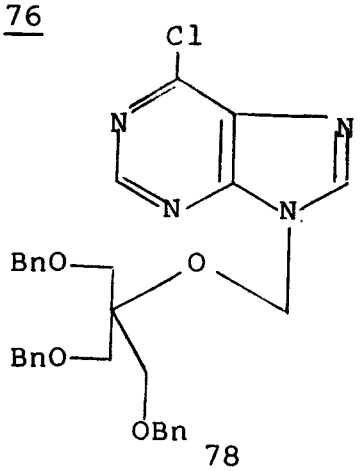

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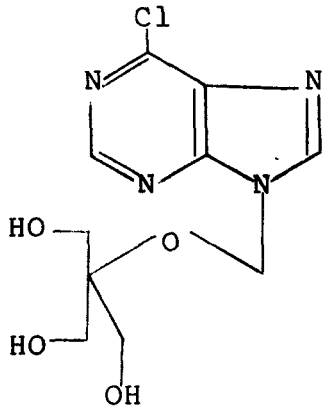

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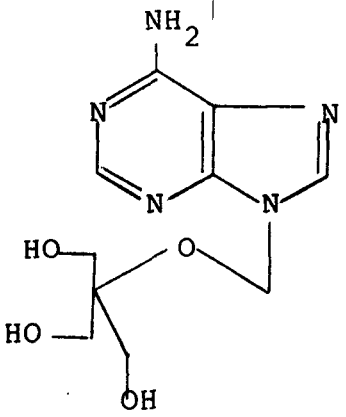

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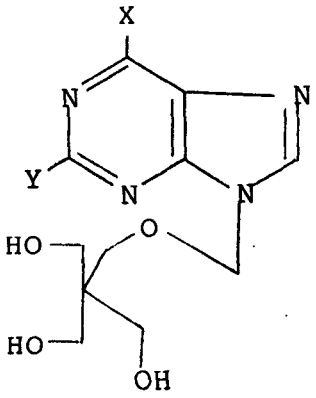
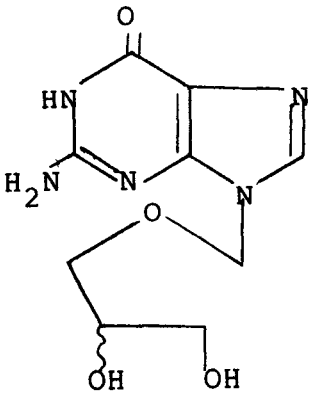


81 a, X=NH_2, Y=H
b, X=OH, Y=NH_2



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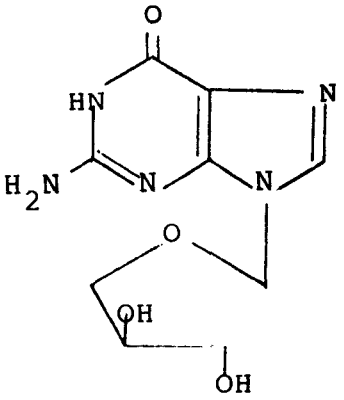
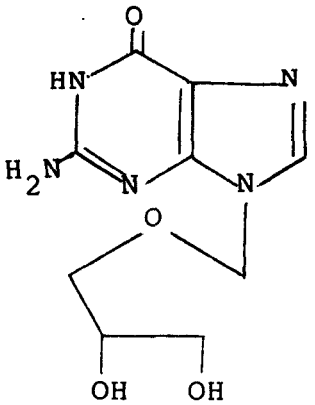


83 (S)-isomer

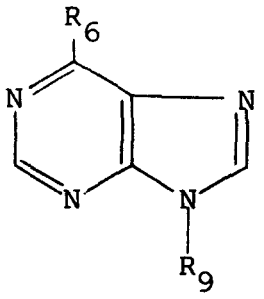


84 (R)-isomer

1.7 ADENOSINE NUCLEOSIDE ANALOGUES

1.7.1 9-(2-Hydroxyethoxymethyl)adenine analogues

Schaeffer and coworkers[75,76] had reported the synthesis and binding characteristics of adenosine deaminase inhibition activity of a number of N_9-alkyl substituted adenine analogues (85-91).

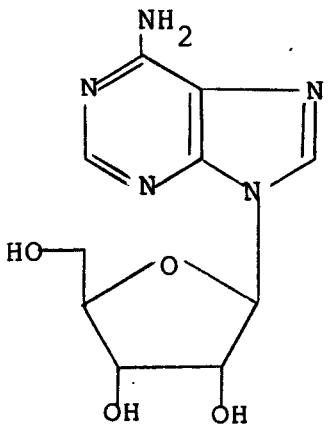


Inhibition of Adenosine deaminase

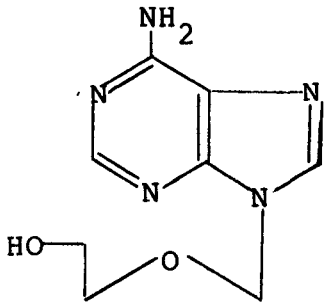
	R_6	R_9
85	NH_2	$HO(CH_2)_2$
86	$NHCH_3$	$HO(CH_2)_2$
87	NH_2	$HO(CH_2)_3$
88	$NHCH_3$	$HO(CH_2)_3$
89	NH_2	$HO(CH_2)_4$
90	$NHCH_3$	$HO(CH_2)_4$
91	Adenosine	

Compounds (85) and (87) were found to be most effective inhibitors and bound to the enzymes twice as tightly as adenosine (91). It was known that 5'-deoxynucleosides of adenine do not undergo deamination with adenosine deaminase unless a properly positioned hydroxyl group is present at C-3' as in 9-(5'-deoxy--D-xylofuranosyl)adenine. This showed that 5'-hydroxyl group plays a role in the deamination reaction. Based on this Schaeffer and coworkers[77] prepared 9-(2-hydroxy ethoxymethyl)adenine (92), which contains the 5'-hydroxyl portion of adenosine (91). However the deoxy compound

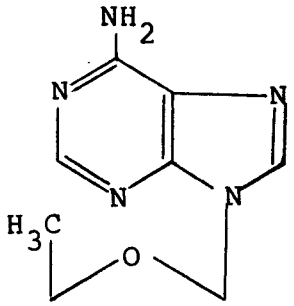
(93) did not show any substrate activity.



91



92

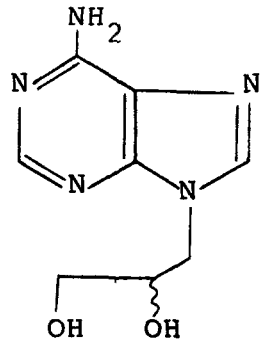


93

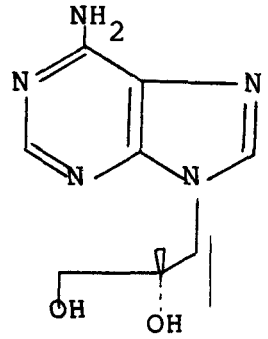
1.7.2 9-(2,3-Dihydroxypropyl)adenine (DHPA) analogues

Antiviral activity of (S)-9-(2,3-dihydroxypropyl) adenine (S-DHPA) had been reported by DeClercq et al[78]. (S)-DHPA inhibited several viruses including vaccinia, HSV-1 and HSV-2, measles and vericular stomatitis. The racimic mixture (RS)-DHPA was as active as the (S)-enantiomers. Which (R)-isomer of DHPA was inactive. (S)-DHPA, strongly inhibited deamination of adenosine and ara-A by adenosine deaminase. It is suggested that nucleoside may inhibit S-adenosyl-L-homocysteine hydrolase[79]. Schaeffer and coworkers[76] had reported that racemic DHPA (94) is a weak inhibitor of adenosine deaminase. Holy[80] had synthesised the (R)- and (S)-enantiomers of (94) and had also prepared[76,78] several other purine

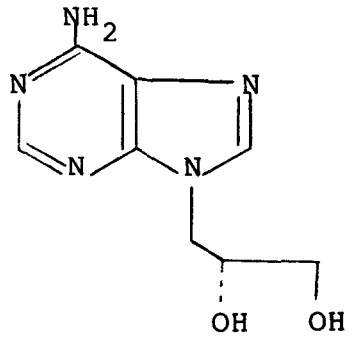
derivatives. However none of the compounds (95-102) showed any significant antiviral activity.



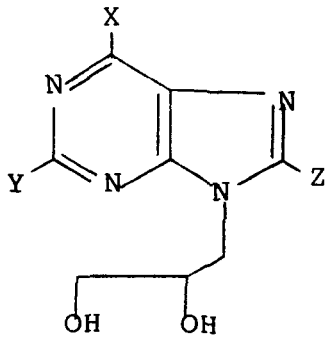
DHPA 94



(S)-(DHPA)

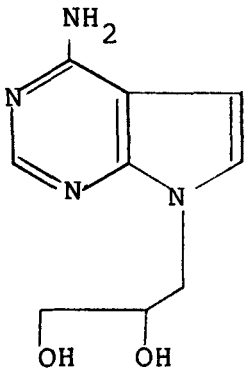


(R)-DHPA

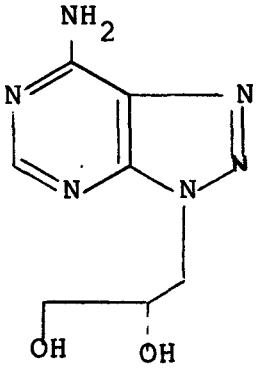


	X	Y	Z
95	$NHCH_3$	H	H
96	$N(CH_3)_2$	H	H
97	OH	H	H
98	SH	H	H
99	NH_2	H	H
100	NH_2	NH_2	H
101	SCH_3	H	H
102	OH	NH_2	H

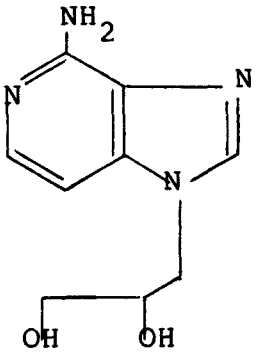
DHPA analogues having different aglycons such as pyrrolo[2,3-d]pyrimidine (103)[81,82], triazolo[4,5-d] pyrimidine (104)[83] did not show any significant activity. The nucleoside (105) showed antiviral activity against vaccimia virus and inhibited S-adenosyl homocystein hydrolase[84].



103



104



105

1.7.3 Erythro-9-[2-hydroxy-3-nonyl]adenine (EHNA)analogues

Schaeffer and coworkers had extensively studied the substrate binding characteristics of adenosine deaminase and found that the binding area for the 9-substituents is a large hydrophobic region[84], a hydroxyl binding site and a specific methyl binding region[85]. In the case of 9-(1-hydroxy-2-alkyl)adenine, the preferred chiral centre for enzyme inhibitor complex formation has the (R)-configuration[86], while in the case of 9-

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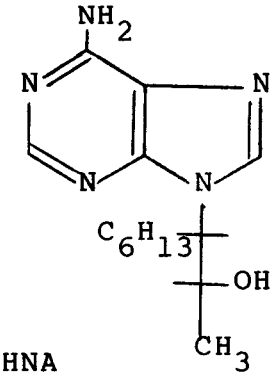
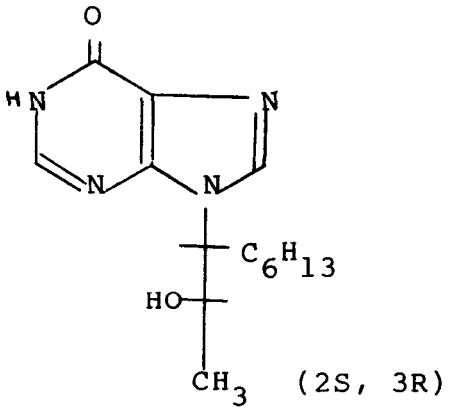
(2-hydroxypropyl)adenine, the chiral centre with (S)-configuration is bound more tightly to the enzyme[85]. Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (106) prepared on the basis of above studies was most potent inhibitor[87].

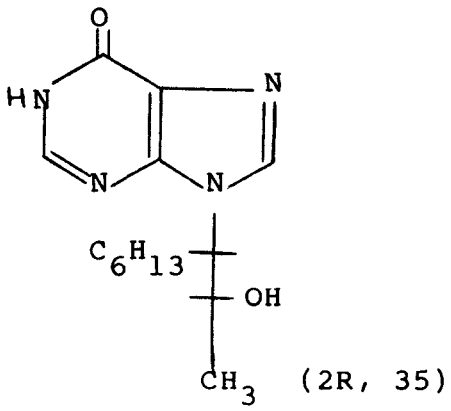


EHNA 106

Structurally related to EHNA, erythro-9-(2-hydroxy-3-nonyl)hypoxanthine (NPT 15392) [a mixture of (107) and (108)] had been reported to have immunopotentiating activity[88,89] . The compound enhances T-cell dependent immune response[90].

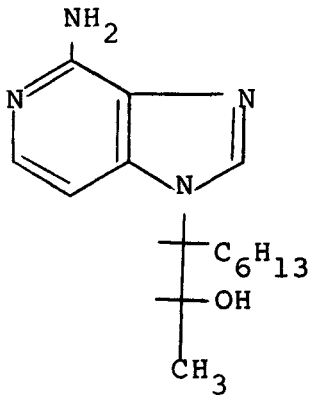


107 (+) erythro

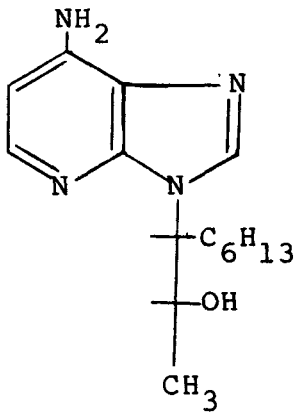


108 (-) erythro

In view of the significant biological activity of EHNA and its hypoxanthine analogues (NPT 15392), Grifantini and coworkers[91] synthesized deaza-analogues of EHNA in order to study the structural requirements of EHNA as an inhibitor of adenosine deaminase. 3-Deaza-EHNA (109) was found to have an inhibitory activity. While 1-deaza-EHNA (110) was found to have less inhibitory activity.



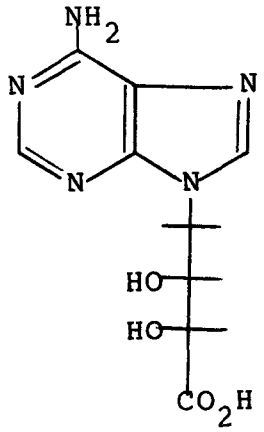
109



110

1.7.4 Eritadenine analogues

Eritadenine (111), a hypolipidemic agent isolated from Japanese edible mushroom Shiitake (Leutinus edodes) had been characterized as 2(R), 3(R)-dihydroxy-4-(9-adenyl)butyric acid. Deoxy eritadenine and 9-carboxy propyl adenine had also been isolated from the same organisms[92-94].

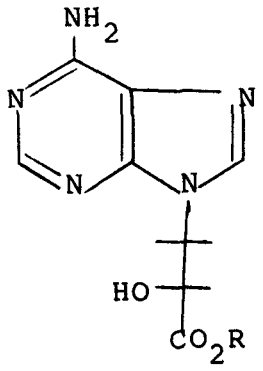


111 Eritadenine

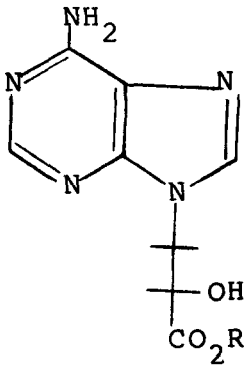
Takeyama and coworkers[95] had synthesized more than 100 derivatives of eritadenine which showed hypocholesterolemic activities. From the structural activity relationship, the carboxylic acid derivatives was found most active with short chain mono hydroxy alcohols. These compounds were found 50 times more active than eritadenine in lowering the serum cholesterol in rate.

The isoamyl esters of eritadenine inhibits protein kinase by acting as a competitive inhibitor of ATP, which indicates that these esters act at the receptor[96] site. Holy and coworkers showed that eritadenine and its analogues as S-adenosyl-L-homocystein hydrolase inhibitors and as antiviral agents[97-104]. So eritadenine and its derivatives inhibits S-adenosyl-L-homocysteine hydrolase.

Since the methyl esters of D-eritadenine is more potent as an antiviral agent than the parent compound. De Clercq and Holy[105] studied various alkyl esters of adenyl-9-yl-2-hydroxypropanoic acid (112).



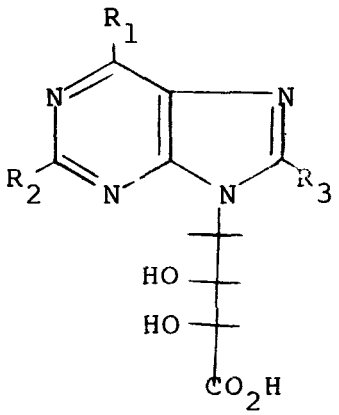
112a



112b

These esters showed broad inhibitory activity against vesicular stomatitis, vaccinia, reo, parainfluenza and measles viruses and they are nontoxic to the host cell at antivirally active concentrations.

Holy et al[104] studied a number of -carboxyl alkyl derivatives of adenine and other purine bases (113-120) for their inhibitory effects on rat liver S-adenosyl-L-homocystein hydrolase and their antiviral activity.



	R_1	R_2	R_3
113	NH_2	H	H
114	NH_2	NH_2	H
115	NH_2	SCH_3	H
116	NH_2	H	Br
117	NHC_2H_5	H	H
118	$N(CH_3)_2$	H	H
119	OH	H	H
120	SCH_3	H	H

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CHAPTER - II

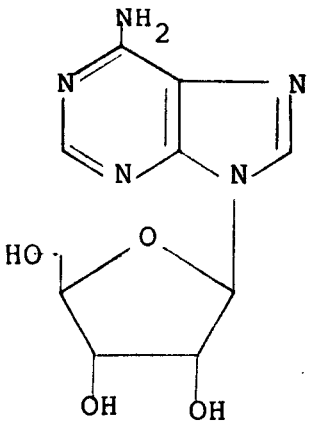
Synthesis of acyclic nucleosides of 4-substituted pyrazolo[3,4-d]pyrimidine and their biological activity

2.1 INTRODUCTION

Adenosine (1), a well known naturally occurring nucleoside, is an effective coronary vasodialator[1,2] and inhibitor of platelets thrombi[3]. It is believed that, when cells are injured, some of their adenosine triphosphate (ATP) breaks down to adenosin diphosphate (ADP), which initiate the aggregation of blood platelets[4]. Adenosine diphosphate on further dephosphorylation by plasma enzymes[5,6] produces adenosine monophosphate (AMP)[7] or adenosine, which exerts its inhibitory action on platelets aggregation[8]. The analogs of adenosine have longer duration of action as compared to adenosine[9,10].

The replacement of ring carbon or nitrogen of purine (2) by a nitrogen or carbon atom gives aza or deaza purines (3,4) ring systems respectively, whereas, interchange of nitrogen at position-7 with carbon at position-8 of purine system (2) affords pyrazolo[3,4-d]pyrimidine (5). A number of deaza and azapurine nucleosides have been synthesized as potential antitumor and antiviral agents[11,12].

Pyrazolo[3,4-d]pyrimidine nucleosides have received considerable attention due to their wide spectrum of biological activities. Allopurinol[13](pyrazolo



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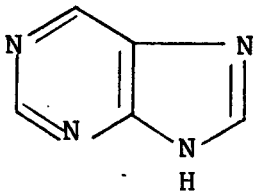

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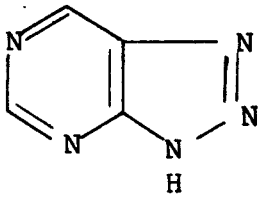

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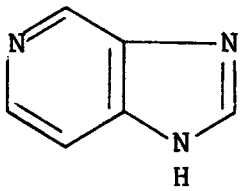

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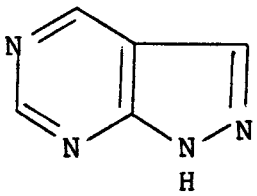

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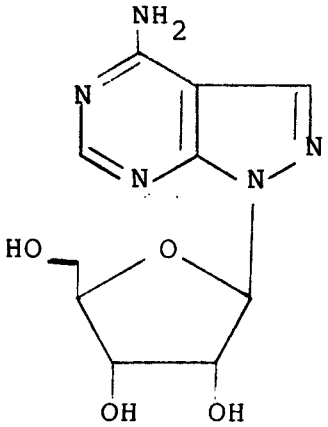

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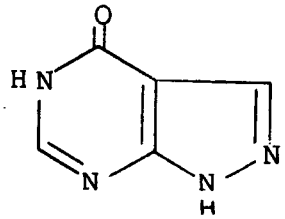

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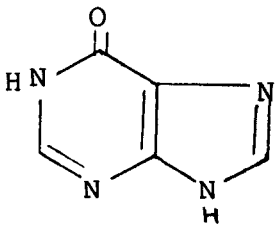

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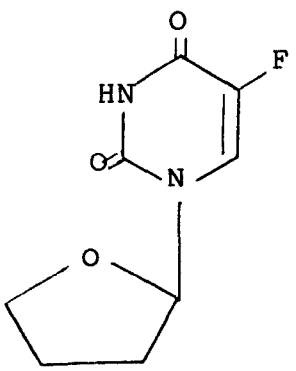
[3,4-d]pyrimidin-4-one) an analogue of hypoxanthine (7) is an inhibitor of purine catabolic enzyme, xanthine oxidase[14] and is used for the treatment of hyperurecemia[15] responsible for gout[16]. 4-Amino pyrazolo[3,4-d]pyrimidinriboside (8) an analogue of adenosine is action against a number of species of Leishmania[17-22].

Several varities of nucleosides have been synthsized in recent years. Some of these nucleosides have shown significant anticancer, antiparasitic and antiviral activities. Few of these are being used as drugs. One of the drawbacks of the biologically active nucleosides is that they are not generally specific to the infected cells. Floraphur[23] (5-fluoro-1-tetrahydro-furyl-uracil) (9), 9-(hydroxy ethoxy methyl)guanine[24](10), 9-cyclohexyl-6-propoxy adenine[25](11) are very active compounds, which are specific to the infected cells. These compounds (9-11) are not true nucleosides.



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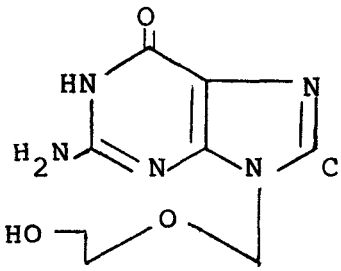

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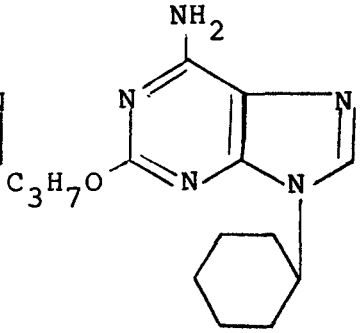

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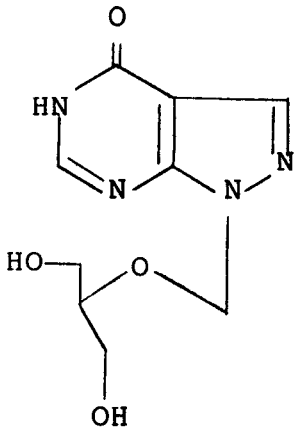
Although the heterocyclic moiety in these compounds is the same as present in the nucleosides of nucleic acids. However, the aglycon part is not the usual D-ribose or D-deoxyribose sugars. The tetrahydrofuryl, cyclohexyl, hydroxy ethoxy methyl moieties attached at position N^9 in these compounds due to their hydrophilic nature may be facilitating the transport of these molecules across the cell membrane. Inside the cell these compounds might be converted into riboside by the action of ribo furanosyl transferase and to nucleosides.

2.2. PRESENT WORK

The above reports prompted us to undertake the synthesis of alicyclic nucleosides of 4-hydroxy and 4-amino pyrazolo[3,4-d]pyrimidines. In the present chapter the synthesis of 1-[2-hydroxy-1-(hydroxy methyl) ethoxy]methyl-4(5H)-oxo pyrazolo[3,4-d]pyrimidine (23), 4-amino-1-[2-hydroxy-1-(hydroxy methyl)ethoxy]methyl pyrazolo[pyrazolo[3,4-d]pyrimidine (26), 1-[2-hydroxy-1-(amino methyl)ethoxy]methyl-4(5H)-oxopyrazolo[3,4-d]pyrimidine (30), 1-[2-hydroxy-1-(amino methyl)ethoxy] methyl-4-amino pyrazolo[3,4-d]pyrimidine (34), 1-[2-hydroxy ethoxy)methyl]-4(5H)-oxopyrazolo[3,4-d]pyrimidine (38), 4-amino-1-[(2-hydroxy ethoxy)methyl]pyrazolo]3,4-d]pyrimidine (40) and their antileishmanial activity

in vivo are reported.



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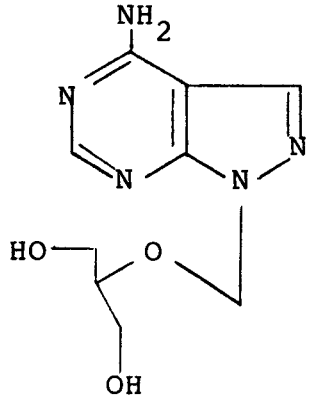

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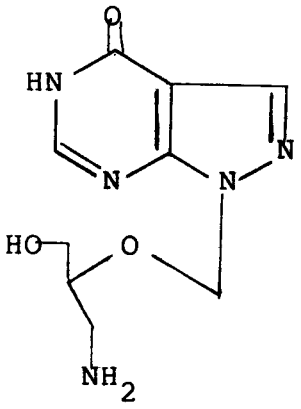

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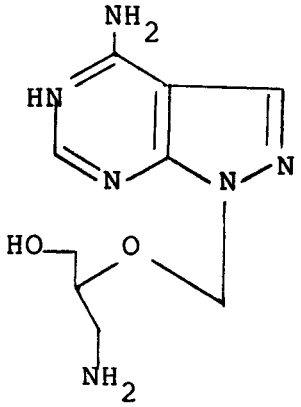

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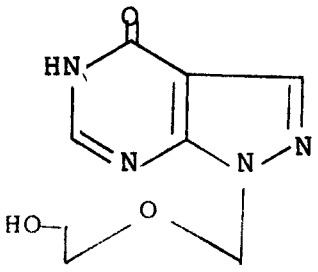

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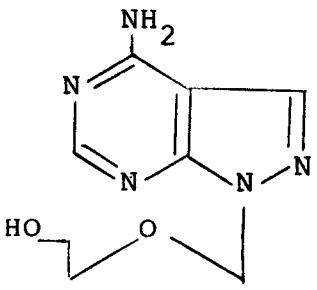

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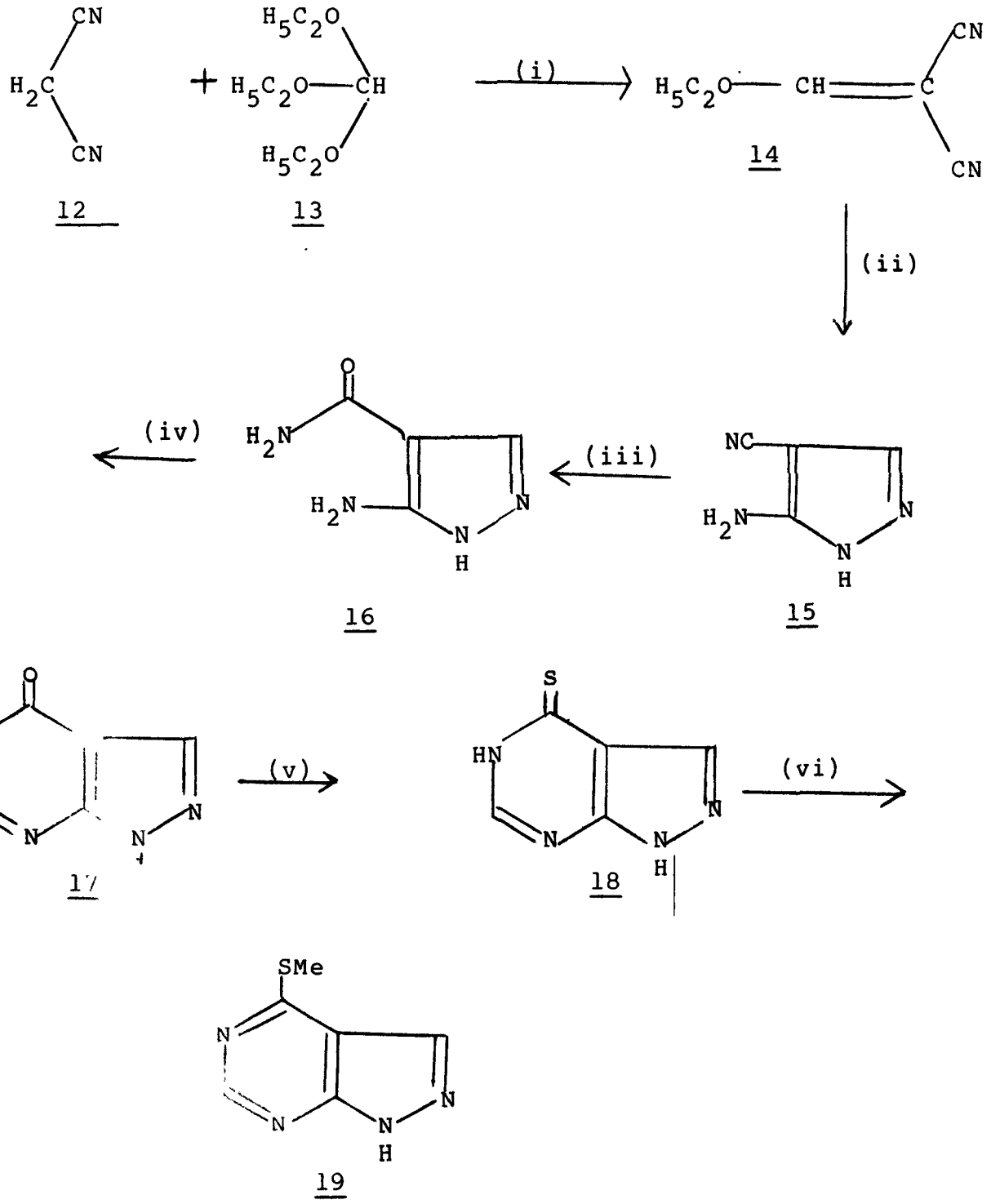
2.3 SYNTHESIS

2.3.1 Synthesis of 4-methylthio pyrazolo[3,4-d]pyrimidine (19)

4-Methylthio pyrazolo[3,4-d]pyrimidine (19) (Scheme 1) the key intermediate with synthesis of 4-substituted-pyrazolo[3,4-d]pyrimidine nucleosides had been synthesized following the method of Robins[13]. Condensation of malononitrile (12) with triethylorthoformate (13) gave ethoxymethylene malononitrile (14) in quantitative yield. Treatment of 14 with hydrazin hydrate yielded 5-amino-4-cyanopyrazole (15) in 30% yield. Hydrolysis of 15 with sulphuric acid afforded 5-amino-pyrazole-4-carboxamide (16) in excellent yield. Cyclisation of 16 with formamide gave 4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (17) in 58% yield. The mass spectrum, elemental analysis and the IR spectrum of 17 were in accordance with the assigned structure. Treatment of 17 with P_2S_5 in refluxing pyridine yielded 4(5H)-thio-pyrazolo[3,4-d]pyrimidine (18), which on methylation with methyliodide under basic conditions yielded 4-methylthio pyrazolo[3,4-d]pyrimidine (19) in 52% overall yield. The pmr spectrum of 19 had a singlet at δ 2.4 for the methylthio group and two singlets at δ 8.2 and δ8.0 for H-6 and H-3 respectively. The mass spectrum of the compound had the base peak at m/z 167 (M^+). Other



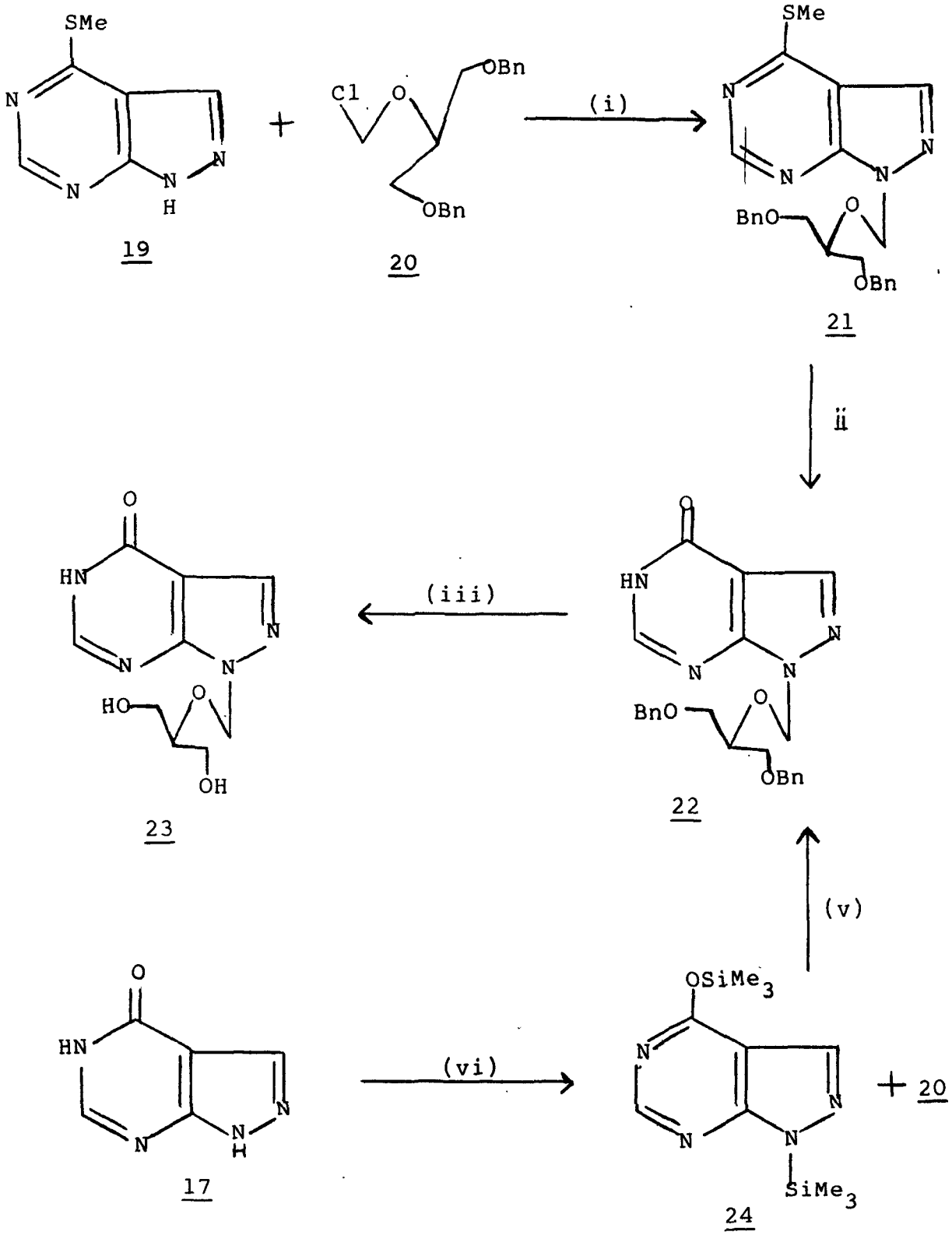
Reagents: (i) $(CH_3CO)_2O$; (ii) $NH_2-NH_2.H_2O$; (iii) H_2SO_4 ; (iv) $HCO-NH_2$; (v) $P_2S_5-C_5H_5N$; (vi) CH_3I-NaOH

significant ions in the spectrum were at m/z 120 ($C_4H_4N_4$) and 93 ($C_4H_3N_3$).

2.3.2 Synthesis of 1-[2-hydroxy-1-(hydroxymethyl) ethoxy]methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (23)

Method-1

Condensation of 4-methylthio pyrazolo[3,4-d]pyrimidine (19) (Scheme 2) with 1,3-dibenzyloxy-2-chloromethyl oxypropane (20) in the presence of Et_3N gave 1-[2-benzyloxy-1-(benzyloxy methyl)ethoxy]methyl-4-methylthio pyrazolo[3,4-d]pyrimidine (21) in 70% yield as an oil. The compund 21 analysed for $C_{24}H_{26}N_4O_3S$ and had molecular in peak at 450 (M^+) in mass spectrum. PMR spectrum of compound 21 had a singlet at δ 5.88 for anomeric proton suggested the attachment of aglycon moiety. Compound 21 on reaction with NaOH gave 4(5H)-oxo derivative (22). Hydrogenolysis of 22 with $PdCl_2$ in H_2 atmosphere furnished the required compound (23) in 55% yield. Compound 23 analysed for $C_9H_{12}N_4O_4$ and had molecular ion peak at 240 (M^+) in its mass spectrum. The ultra violet absorption of the compound 23 (λ max 251, 206 nm). The site of alkylation in compound 23 was established by correlating the UV absorption pattern with Table-1[26]. Compound 23 shows similar UV absorption pattern as allopurinol-1-riboside, reflecting 23 to be N-1

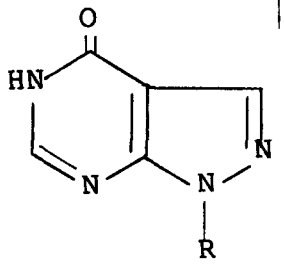


Reagents: (i) DMF-Et_3N ; (ii) NaOH-Dioxan
(iii) $PdCl_2$-H_2 ; (iv) HMDS/$(NH_4)_2SO_4$;
(v) C_6H_6

Scheme 2

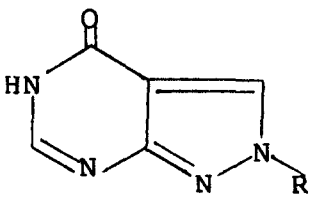
Table 1: UV Absorption maxima of substituted pyrazolo [3,4-d]pyrimidine derivatives

N-1-isomer



Compd. No.	R	λ max (nm) pH 7	λ max (nm) pH 11
23	$-CH_2-O-CH(CH_2OH)-CH_2OH$	251	270
30	$-CH_2-O-CH(CH_2-NH_2)-CH_2-OH$	250	271
38	$-CH_2-O-CH_2-CH_2-OH$	250	271
41	-Me	249	270
42	-ribose	252	271

N-2-isomer



43	-Me	255	280
44	-ribose	261	284

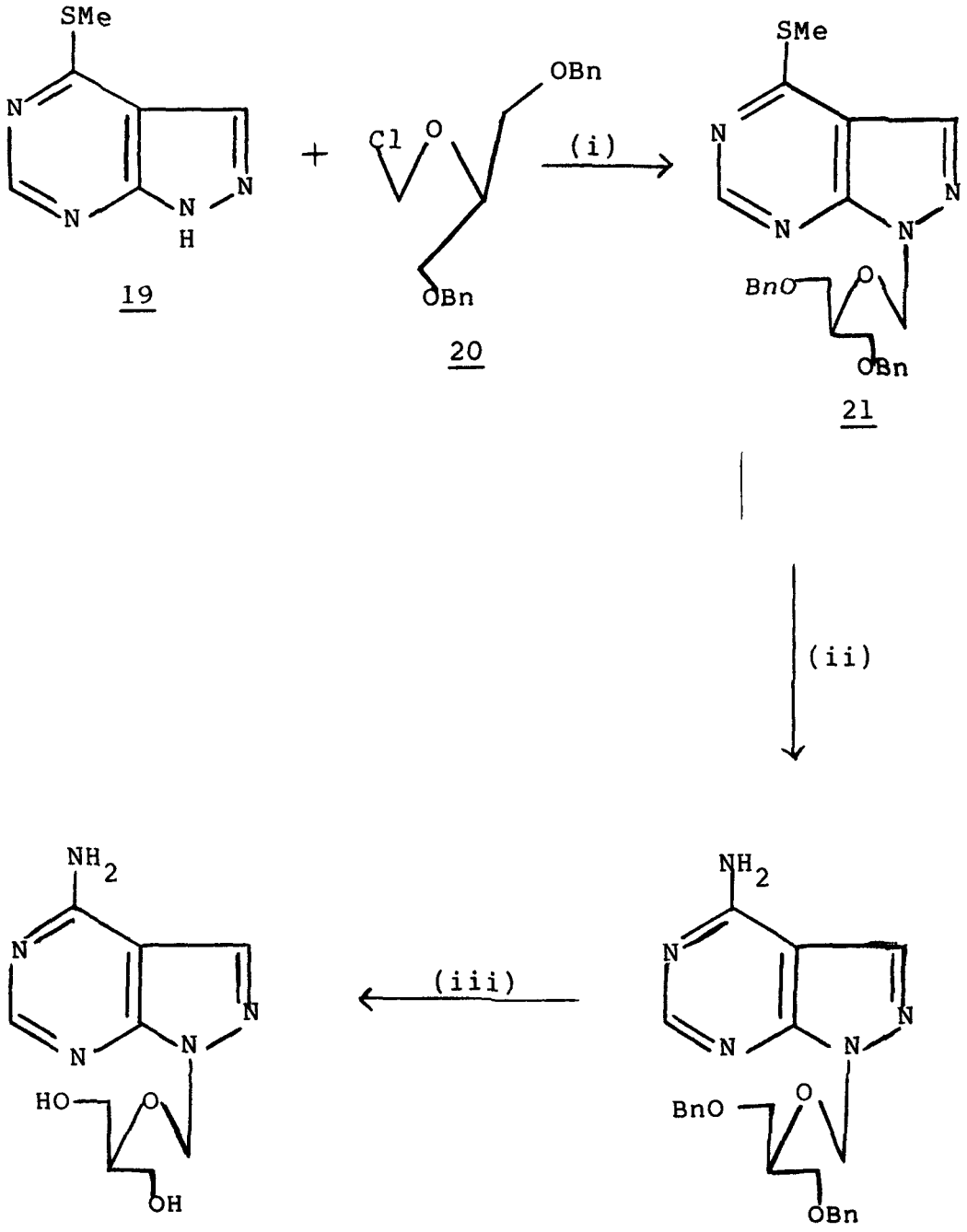
isomer. PMR spectrum of the compound 23 had two singlets at δ 7.95 and δ 7.9 for H-6 and H-3 respectively in the heterocyclic moiety. In the spectrum of 23 a two singlet at δ 5.75 for anomeric proton suggested the attachment of aglycon moiety.

Method-2

The reaction of 4-hydroxy-pyrazolo[3,4-d]pyri- (17) with hexamethyldisilazane (HMDS) in the presence of $(NH_4)_2SO_4$ in a catalytic amount gave silylated derivative (24). The condensation of the 24 (Scheme 2) with 1,3-dibenzyloxy-2-chloromethyloxy propane (20) in refluxing benzene gave compound (22), which on hydrogenolysis with $PdCl_2$ furnished (23). The compound 23 obtained by this procedure was identical in all respect with the compound made by Method-1.

2.3.3 Synthesis of 1-[2-hydroxy-1-(hydroxymethyl) ethoxy]methyl-4-aminopyrazolo[3,4-d]pyrimidine (26)

Condensation of 4-methylthio pyrazolo[3,4-d]pyrimidine (19) (Scheme 3) with 20 gave 21. Compound 21 on reaction with ammonia under pressure at elevated temperature afforded (25) in 50% yield as an oil. Compound 25 analysed for $C_{23}H_{25}N_5O_3$ had a molecular ion peak at 419 (M^+) in mass spectrum. PMR spectrum of the compound 25 had a two proton singlets at δ 5.8 for anomeric



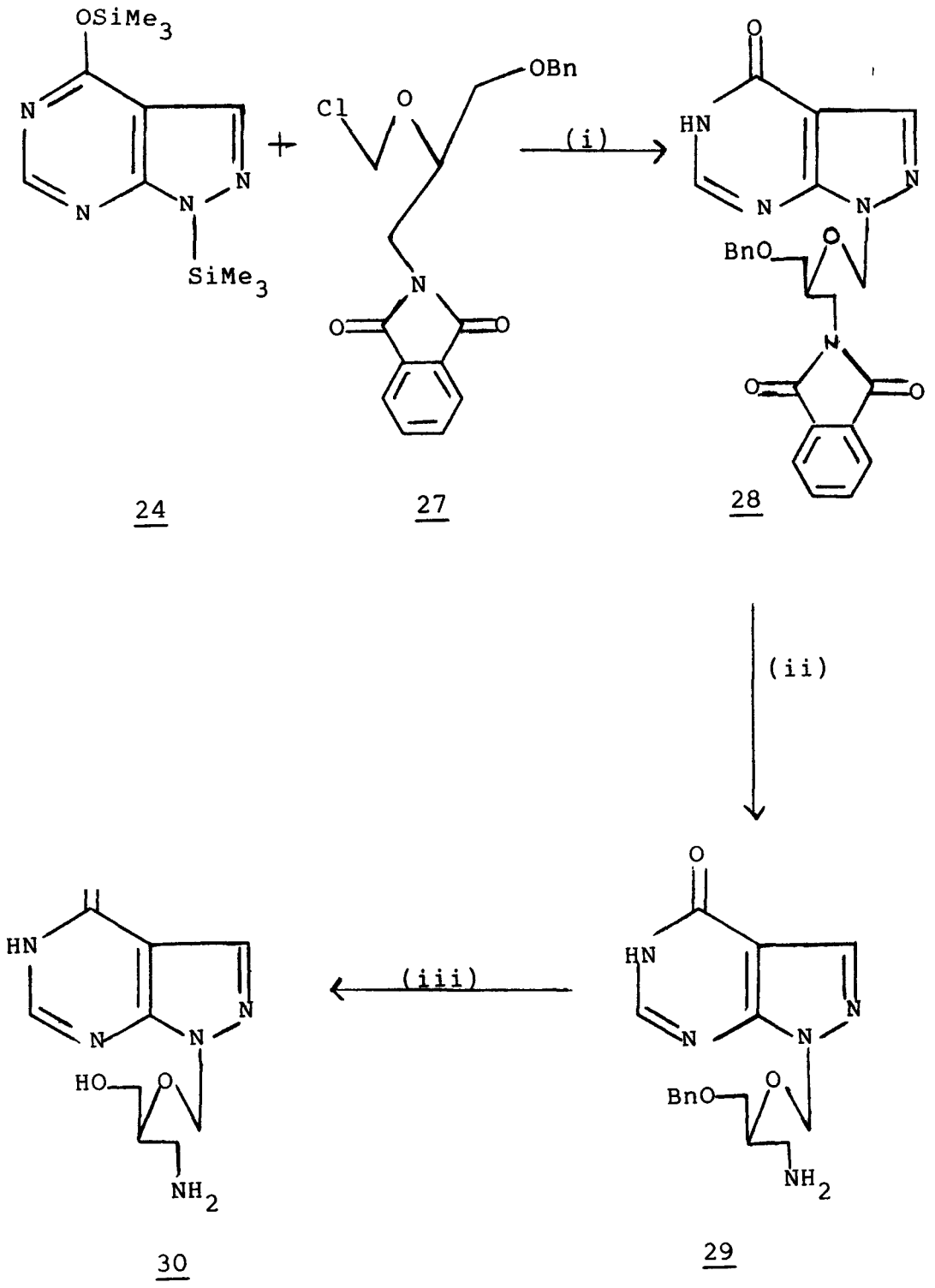
Reagents: (i) DMF-Et_3N ; (ii) MeOH-NH_3 ;
(iii) $PdCl_2$-H_2 *Bn = $-CH_2Ph$

Scheme 3

protons suggested the attachment of aglycon moiety. Compound25 on hydrogenolysis with $PdCl_2$ in H_2 atmosphere. furnished the required compound (26) in 40% yield. The compound 26 analysed for $C_9H_{13}N_5O_3$ had molecular ion peak at 239 (M^+) in mass spectrum. In the infrared spectrum the N-H stretching was at 3100 cm^{-1}. PMR spectrum of the compound 26 had a singlet at δ8.1 for H-6and a singlet at δ 7.5 for H-3 of the heterocyclic moiety. For anomeric proton, a singlet at δ 5.7 suggested the attachment of aglycon moiety.

2.3.4 Synthesis 1-[2-hydroxy-1-(aminomethyl)ethoxy] methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (30)

Condensation of bis(trimethylsilyl) derivative of 4-hydroxy pyrazolo[3,4-d]pyrimidine (24) (Scheme 4) with 2-chloromethoxy-1-benzyloxy-3-phthaloylimidopropane[27] (27) in refluxing benzene gave 1-[2-benzyloxy-1-(phthaloylimidomethyl)ethoxy]methyl-4(5H)-oxo-pyrazolo [3,4-d]pyrimidine (28). The pmr spectrum of the compound 28 had a singlet at δ8.5 for H-6 and δ8.2 for H-3 in the heterocyclic moiety. While in the aglycon moiety it had a broad singlet at δ 7.6-7.8 for 5H for Ar-H and for aromatic protons it had a broad singlet at δ7.3. Compound 28 had a singlet at δ 5.7 for anomeric protons suggested the attachment of aglycon moiety.



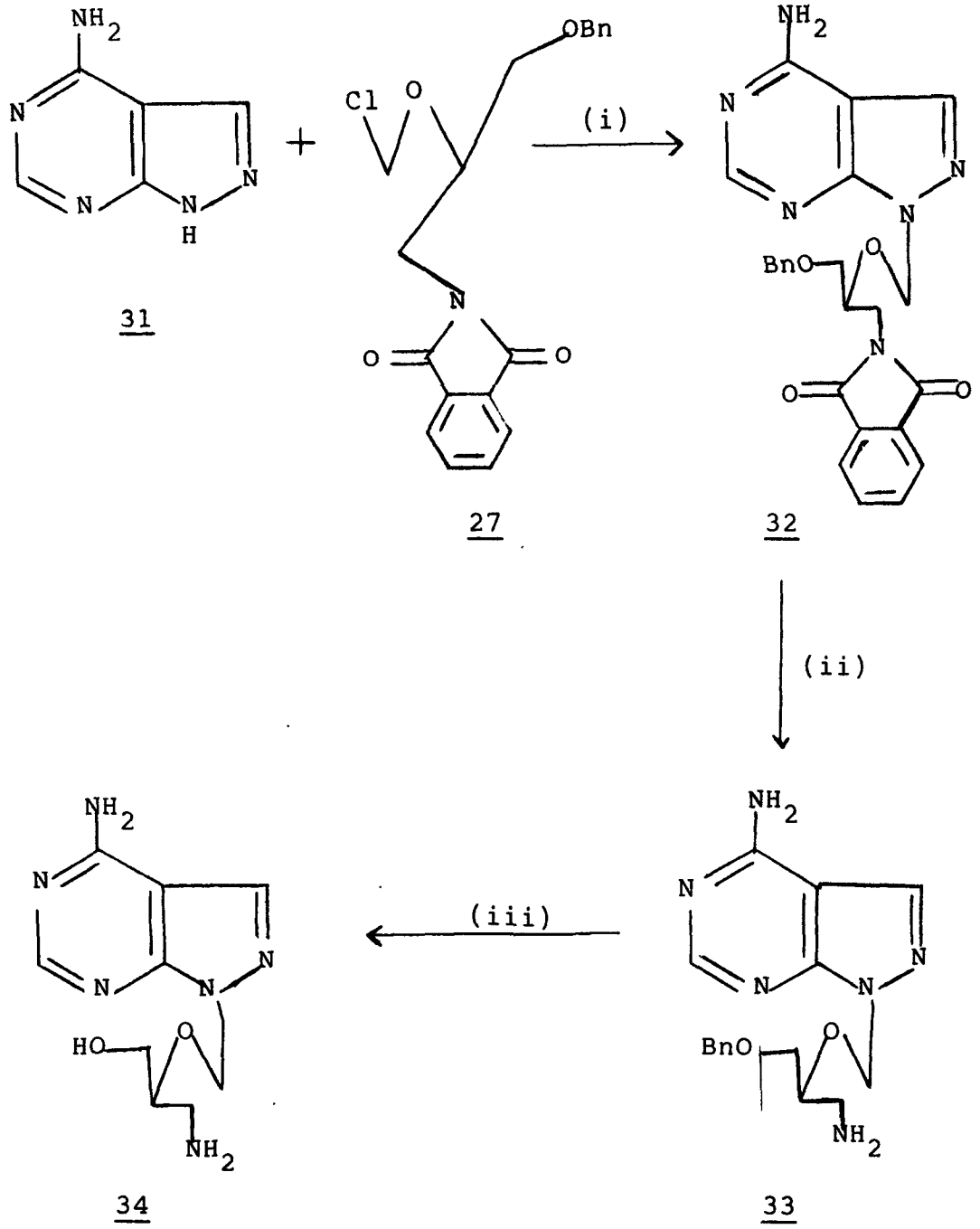
Reagents: (i) C_6H_6 ; (ii) NH_2-NH_2.H_2O,MeOH ;
(iii) $PdCl_2$-H_2

Scheme 4

The protecting phthaloyl group was removed by the reaction of 28 with hydrazin hydrate at 0° temperature in EtOH to yield (29) in 40% yield as an oil. Which on debenzylation with $PdCl_2$-H_2 afforded required compound (30) in 30% yield. Compound 30 analysed for $C_9H_{13}N_5O_3$ had molecular ion peak at 239 (M^+) in mass spectrum. The ultra violet absorption of the compound 30 (λmax 250, 206 nm) shows the similar UV absorption pattern as in the case of allopurinol-1-riboside, refluting 30 to be N-1 isomer. PMR spectrum of the compound 30 had two singlets at δ8.0 and δ7.8 for H-6, H-3 respectively for heterocyclic moiety and had a singlet at δ5.8 for anomeric protons suggested the attachment of aglycon moiety.

2.3.5 Synthesis of 1-[2-hydroxy-1-(aminomethyl)ethoxy] methyl-4-amino pyrazolo[3,4-d]pyrimidine (34)

Condensation of 4-amino pyrazolo[3,4-d]pyrimidine (31) (Scheme 5) 2-chloromethoxy-1-benzyloxy-3-phthaloylimido propane (27) in dry DMF gave 1-[2-benzyloxy-1-(phthaloylimidomethyl)ethoxy]methyl-4-amino pyrazolo[3,4-d]pyrimidine (32). The protecting phthaloyl group was removed by treatment of 32 at 0° with hydrazine hydrate to yield (33) as an oil in 40% yield. Debenzylation of 33 with $PdCl_2$-H_2 atmosphere afforded the required compound (34) in 58% yield. The compound 34 analysed for $C_9H_{14}N_6O_2$ had molecular ion peak at 238



Reagents: (i) DMF, NaH ; (ii) NH_2-NH_2.H_2O-MeOH ; (iii) $PdCl_2$-H_2

Scheme 5

(M^+ +1). PMR spectrum had two singlets at δ 8.4 and δ8.1 for H-6 and H-3 respectively and also had a singlet at δ 5.9 for anomeric protons of aglycon moiety.

2.3.6 Synthesis of 1-(hydroxyethoxy)methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (38)

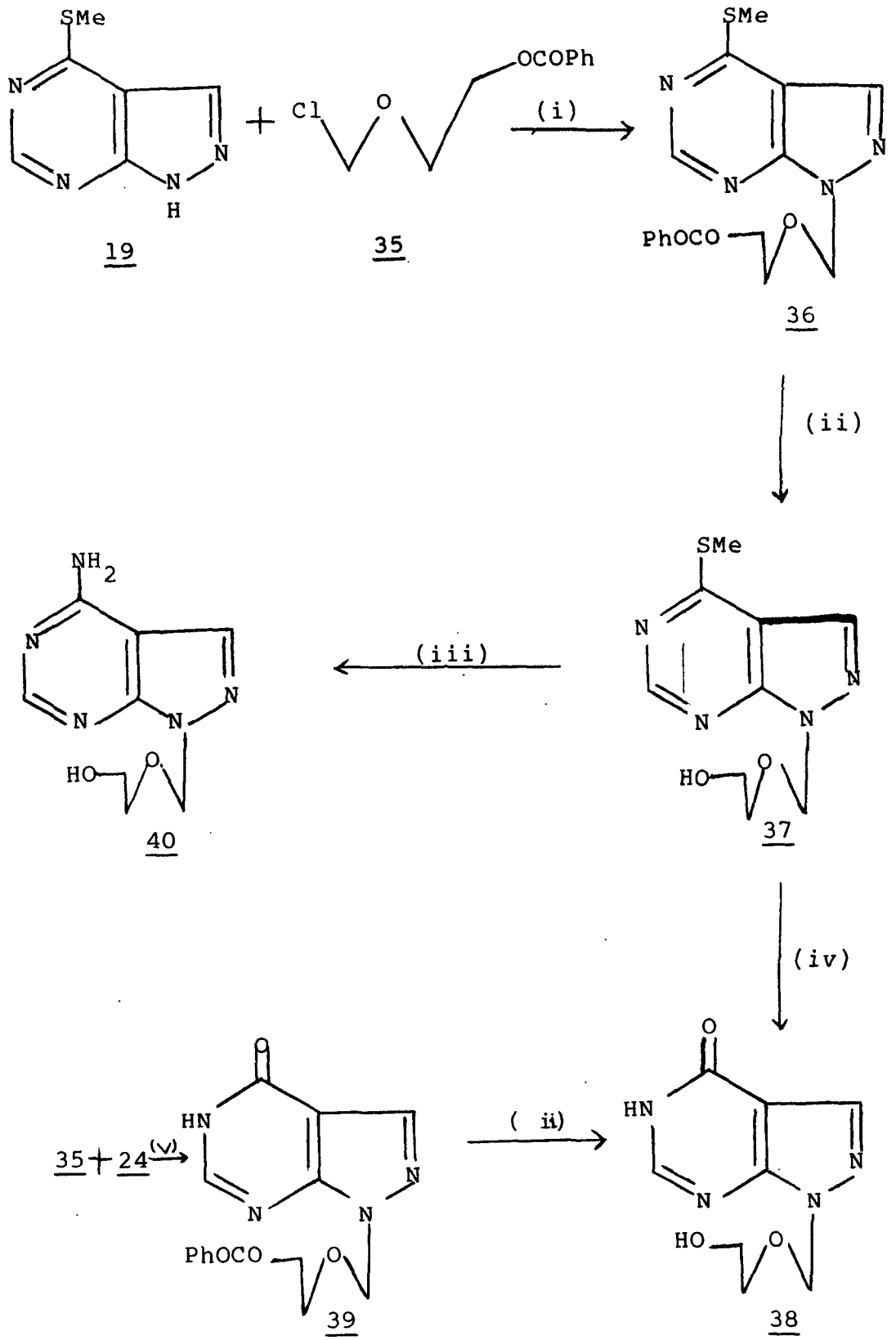
Method-1

Condensation of 4-methylthio pyrazolo[3,4-d]pyrimidine (19) (Scheme 6) with benzoyloxy ethoxy methylene chloride in the presence of Et_3N gave 1-(benzoyloxyethoxy) methyl-4-methylthio pyrazolo[3,4-d]pyrimidine (36) in 60% yield. Deblocking of the protected nucleoside 36 with methanolic ammonia at ambient temperature afforded 37 in 55% yield. Treatment of 37 with aq. KOH gave 1-(hydroxyethoxy)methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (38) in 30% yield. Compound 38 analysed for $C_8H_{10}N_4O_3$ had molecular ion peak at m/z 210 (M^+). Ultra violet absorption of the compound 38 (λ max 250.6, 207.8 nm) suggested position N-1 the site of alkylation.

PMR spectrum of the compound 38 had two singlets at δ 7.95 and δ7.9 for H-6 and H-3 respectively and a singlet at δ5.7 for anomeric protons.

Method-2

Condensation of 24 with 35 in refluxing benzene gave 1-(benzoyloxyethoxy)methyl-4(5H)-oxo-pyrazolo[3,4-d]



19 35 36 37 38 39 40

Reagents: (i) DMF, Et_3N; (ii) $MeOH-NH_3$, R.T.; (iii) $MeOH-NH_3$, Δ ; (iv) NaOH-Dioxane (v) C_6H_6

pyrimidine (39) in 58% yield. Treatment of 39 with methanolic NH_3 furnished required compound (38). The compound 38 obtained by this procedure was identical in all respect with the compound made by the Method-1.

2.3.7 Synthesis of 1-(hydroxyethoxy)methyl-4-amino pyrazolo[3,4-d]pyrimidine (40)

Treatment of compound 37 (Scheme 6) with NH_3 at eluated temperature in a steel bomb gave 1-(hydroxyethoxy)methyl-4-amino pyrazolo[3,4-d]pyrimidine (40) in 50% yield. Compound 40 analysed for $C_8H_{11}N_5O_2$ had a molecular ion peak at 209 (M^+) in mass spectrum. PMR spectrum of the compound had two singlets at δ 8.2 and δ 8.1 for H-6 and H-3 protons respectively and a singlet at δ 5.7 for anomeric protons.

2.4 EXPERIMENTAL PROCEDURE

Melting points were taken with Buchi capillary apparatus (silicon bath) and were uncorrected. UV spectra were recorded on a Perkin Elmer-202 spectrophotometer (λ max in nm). IR spectra on a Perkin Elmer 157 grating infracord (λ max cm^{-1}). The PMR spectra were recorded on a Perkin Elmer 360L at 60 MHz (chemical shift δ scale). The compounds were routinely checked on silica gel plates and spots were located either under UV lamp or by iodine vapours or by spraying with 10% sulphuric acid in ethanol followed by heating at 100°C or by spraying with a mixture of p-anisaldehyde (2%) and sulphuric acid (10%) in ethanol followed by heating at 100°C. Evaporation of solution were carried out under reduced pressure with the bath temperature below 40°C.

1-[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl-4-methyl thio pyrazolo[3,4-d]pyrimidine (23)

A mixture of 4-methylthio pyrazolo[3,4-d]pyrimidine (19) (3.o g, 18 mmol), DMF (30 ml) and Et_3N (15 ml) was stirred at ambient temperature. To the mixture was added a solution of 1,3-dibenzyloxy-2-chloromethoxy propane (20) (6.0 g, 18 mmol) in DMF (10 ml) and the mixture was stirred for 14 hr. The excess of reagent

and solvent were removed at reduced pressure. The residue was taken in EtOAc, washed with H_2O (2x100 ml), dried (Na_2SO_4) and the solvent removed. The product thus obtained was chromatographed on SiO_2 column. Elution of the column with $CHCl_3$gave 21, as an oil (3.9 g, yield 70%); MS (m/z): 450 (M^+); PMR($CDCl_3$): 8.7 (s, 1H, H-6), 7.9 (s, 1H, H-3), 7.2 (m, 10H, Ph-H), 5.88 (s, 2H, H-1), 4.4-4.3 (each s, 4H, CH_2Ph), 3.9 (m, 1H, H-4'), 3.6-3.3 (m, 4H, H-3', H-5'), 2.6 (m, 3H, SCH_3); (Found: C, 64.1; H, 5.8; N, 12.5. $C_{24}H_{26}N_4O_3S$ requires C, 64.1, H, 5.9; N, 12.5%.)

1-[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (22)

Method-1

Compound 21 (1.5 g, 3 mmol) in dioxane (30 ml) was refluxed with aqueous 20% KOH (30 ml) for 12 hr. The resulting mixture was cooled, neutralised with AcOH and the solvent removed under reduced pressure. The residue was taken in $CHCl_3$, washed with water, dried (Na_2SO_4) and concentrated. The product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (96:4, v/v) gave (22) as an oil, (0.4 g, yield 30%); MS (m/z): 420 (M^+); IR(neat): 1710 (C=O); PMR($CDCl_3$): 8.4 (s, 1H, H-6), 8.1 (s, 1H, H-3), 7.2 (m, 10H, PhH), 5.75 (s, 2H, H-1'), 4.4 (bs,

4H, H-3', 5'), 3.7-4.1 (m, 1H, H-4'), 3.3-3.5 (d, 4H, -OC<u>H</u>$_2$); (Found: C, 65.76; H, 5.75; N, 13.3. $C_{23}H_{24}N_4O_4$ requires C, 65.8; H, 5.8; N, 13.5%).

Method-2

A mixture of pyrazolo[3,4-<u>d</u>]pyrimidine-4(5H)-one (17) (2.0 g, 15 mmol), hexamethyldisilazane (8 ml), dry toluene (50 ml) and $(NH_4)_2SO_4$ (150 mg) was refluxed for 24 hr. The excess of reagent and solvent from the resulting mixture were removed at reduced pressure to give <u>24</u>, which was used as such for further reaction 1,3-dibenzyloxy-2-chloromethyloxy propane (20) (6.0 g, 19 mmol) was added to <u>24</u> in dry benzene and refluxed for 12 hr. It was then cooled and filtered. The solvent from the filtrate was removed under reduced pressure. The residue was taken in $CHCl_3$, washed with aq. $NaHCO_3$ (2x100 ml), set aq. NaCl solution (2x100 ml), H_2O, dried (Na_2SO_4) and the solvent evaporated. The product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (96:4, v/v) gave (22) (1.1 g, yield 55%) as an oil.

1-[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl-4(5H)-oxo-pyrazolo[3,4-<u>d</u>]pyrimidine (23)

A mixture of <u>22</u> (0.4 g, 95 mmol), $PdCl_2$ (50 mg) and MeOH (20 ml) was shaken in H_2 atmosphere (45 lbs pressure) for 14 hr and filtered. The filtrate was

passed through ion exchange resin (IR-45, $\bar{O}H$ form) and eluted with MeOH. The solvent from the eluate was removed. The product was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (80:20, v/v) afforded (23) (0.2 g, 55% yield); m.p.162° (EtOH); MS (m/z): 240 (M^+); IR(KBr): 1680 (C=O); UV(MeOH) λmax: 251, 206; (NaOH): 270, 213; (Hcl): 250, 208; PMR($CDCl_3$+DMSO-d_6): 7.95 (s, 1H, H-6), 7.9 (s, 1H, H-3), 5.75 (s, 2H, H-1'), 3.55-3.7 (m, 1H, H-4'), 3.3-3.5 (m, 4H, H-3',5'); (Found: C, 40.0; H, 5.0; N, 23.3. $C_9H_{12}N_4O_4$ requires C, 40.1; H, 5.2; N, 23.4%).

1-[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl-4-amino pyrazolo[3,4-d]pyrimidine (25)

Compound 21 (2.0 g, 4 mmol) and methanolic ammonia (25 ml) was heated in steel bomb at 110°C for 14 hr. Solvent and excess of ammonia were removed and the residue were chromatographed on SiO_2 column. Elution with $CHCl_3$:MeOH (96:4, v/v) gave (25) as an oil (1 gm, yield 50%); MS: 419 (M^+); PMR($CDCl_3$): 8.3 (s, 1H, H-6), 7.8 (s, 1H, H-3), 7.3 and 7.2 (each s, 5H, Ar-H), 5.8 (s, 2H, H-1'), 4.5 and 4.4 (each s, 2H, -OCH_2), 4.0 (m, 1H, H-4'), 3.7-3.3 (m, 4H, H-3', 5'); (Found: C, 65.9; H, 6.0; N, 16.7 $C_{23}H_{25}N_5O_3$ requires C, 65.8; H, 6.1; N, 16.8%).

1-[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl-4-amino pyrazolo[3,4-d]pyrimidine (26)

Compound 25 (0.8 g, 1.9 mmol), $PdCl_2$ (100 mg) and MeOH (30 ml) was shaken in H_2 atmosphere (45 lbs pressure) for 14 hr and filtered. The filtrate was passed through ion exchange resin (IR-45, $\bar{O}H$ form) and eluted with MeOH. The solvent from the eluate was removed. The product was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (80:20, v/v) afforded (26) (0.3 g, yield 40%); m.p.182°C; MS (m/z) 239 (M^+); IR(KBr): 3100 cm^{-1} (N-H); PMR($CDCl_3$+DMSO-d_6): 8.1 (s, 1H, H-6), 7.5 (s, 1H, H-3), 5.7 (s, 2H, H-1'), 4.3 (m, 1H, H-4'), 3.1-3.5 (m, 4H, H-3', 5'); (Found: C, 45.2; H, 5.5; N, 29.3 $C_9H_{13}N_5O_3$ requires C, 45.2; H, 5.6; N, 28.7%).

1-[2-Benzyloxy-1-(phthaloyliminomethyl)ethoxy]methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (28)

A mixture of 24 (3.0 g, 20 mmol) and 2-chloromethyloxy-1-benzyloxy-3-phthaloylimido propane (27) (9.0 g, 25 mmol) and dry benzene (150 ml) was stirred at ambient temperature for 2 hr and then refluxed for 14 hr. The solvent from the resulting mixture was removed. The residue was taken in $CHCl_3$, washed with $NaHCO_3$ (2x 150 ml), NaCl (2x100 ml), H_2O, dried (Na_2SO_4) and the

solvent removed. The product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (98:2, v/v) gave (28), as an oil (2.5 g, yield 52%); MS (m/z): 459 (M^+); PMR($CDCl_3$): 8.5 (s, 1H, H-6), 8.2 (s, 1H, H-3), 7.6-7.8 (bs, 5H, Ar-H), 7.3 (bs, 4H, Ar-H), 5.7 (s, 2H, H-1'), 4.4 (s, 2H, $-OCH_2Ph$), 4.0-4.2 (m, 1H, H-4'), 3.4-3.8 (m, 4H, H-3', H-5').

1-[2-Benzyloxy-1-(aminomethyl)ethoxy]methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (29)

A mixture of 28 (2.0 g, 4 mmol), MeOH (60 ml) and hydrazine hydrate (6 ml, 98%) was kept at 0° for 12 hr. The solvent from the resulting mixture was removed at reduced pressure. The residue extracted with $CHCl_3$ the solvent removed. The crude product was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (95:5, v/v) gave (29) as an oil, (0.8 g, yield 40%); MS (m/z): 329 (M^+); PMR($CDCl_3$): 8.2 (s, 1H, H-6), 7.9 (s, 1H, H-3), 7.2 (s, 5H, Ar-H), 5.75 (s, 2H, H-1'), 4.2 (s, 2H, $-CH_2Ph$), 3.9-3.6 (m, 1H, H-4'), 3.4-3.2 (m, 4H, H-3', 5').

1-[2-Hydroxy-1-(aminomethyl)ethoxy]methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (30)

A mixture of 29 (0.6 g, 1.8 mmol), MeOH (30

ml), $PdCl_2$ (0.09 g) was shaken under H_2 atmosphere (45 lbs pressure) for 12 hr and filtered. The filtrate was passed through ion exchange resin (IR-45, $\bar{O}H$ form). The solvent from the eluate was removed and the product was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (80:20, v/v) gave (30) (0.2 g, yield 30%); m.p.202° (EtOH); MS (m/z): 239 (M^+); UV (MeOH) nm: 250, 206; (NaOH): 271.6, 211.8; (HCl): 250.2, 207.2; PMR($CDCl_3$+DMSO-d_6): 8.0 (s, 1H, H-6), 7.8 (s, 1H, H-3), 5.8 (s, 2H, H-1'), 3.0-2.5 (m, 4H, H-3', 5'), 2.6-2.4 (m, 1H, H-4'); (Found: C, 45.1; H, 5.5; N, 29.3 $C_9H_{13}N_5O_3$ requires C, 45.2; H, 5.4; N, 29.3%).

1-[2-Benzyloxy-1-(phthaloylimidomethyl)ethoxy]methyl-4-amino pyrazolo[3,4-d]pyrimidine (32)

A mixture of 4-amino pyrazolo[3,4-d]pyrimidine (31) (2.0 g, 0.015 mmol), dry DMF (25 ml) and NaH (0.5 g, 0.02 mmol) was stirred at ambient temperature for 1 hr. To it was added 2-chloromethyloxy-1-benzyloxy-3-phthaloylimidoglycerol (27) (8.0 g, 0.02 mmol) in dry DMF (15 ml) and stirred for 2 hr and then refluxed for 12 hr. Resulting mixture was cooled, H_2 was added and extracted with $CHCl_3$, washed with H_2O (2x150 ml), dried (Na_2SO_4) and concentrated in vacuo. The crude product thus obtained was chromatographed over SiO_2 column. Elut-

ion of the column with $CHCl_3$:MeOH (98:2, v/v) gave (32) as an oil (1.9 g, yield 55%); MS (m/z): 458 (M^+); PMR($CDCl_3$): 8.0 (s, 1H, H-3), 7.7 (s, 1H, H-6), 7.25 (s, 5H, Ar-H), 7.4-7.6 (d, 4H, Ar-H), 5.6 (s, 2H, H-1'), 4.9 (s, 2H, -O-CH_2Ph), 4.1-4.3 (m, 1H, H-4'), 3.4-3.6 (m, 4H, H-3', 5').

1-[2-Benzyloxy-1-(aminomethyl)ethoxy]methyl-4-amino pyrazolo[3,4-d]pyrimidine (33)

A mixture of 32 (1.2 g, 0.003 mmol), MeOH (50 ml) and hydrazin hydrate (5 ml, 0.1 mmol) was kept at $0^{o}$for 12 hr. The solvent and excess of reagent were removed under reduced pressure. The residue was taken in $CHCl_3$ (50 ml) and concentrated. The crude product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (95:5) gave (33) as an oil (0.5 g, yield 40%); MS (m/z): 328 (M^+); PMR ($CDCl_3$+DMSO-d_6): 8.25 (s, 1H, H-6), 8.15 (s, 1H, H-3), 7.2 (s, 5H, Ar-H), 5.8 (s, 2H, H-1'), 4.35 (s, 2H, -CH_2Ph), 4.1-4.3 (m, 1H, H-4'), 3.5-3.6 (m, 4H, H-3', 5'); (Found: C, 58.5; H, 6.1; N, 25.6 $C_{16}H_{20}N_6O_2$ requires C, 58.6; H, 6.3; N, 25.7%).

1-[2-Hydroxy-1-(aminomethyl)ethoxy]methyl-4-amino pyrazolo[3,4-d]pyrimidine (34)

A mixture of 33 (0.4 g, 1.2 mmol) and $PdCl_2$

(60 mg), MeOH (25 ml) was shaken at 45 lbs pressure under H_2 atmosphere. The catalyst was filtered, and the filtrate was neutralised with resin (IR-45, $\bar{O}H$ form) and the solvent removed. The crude product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (80:20, v/v) afforded (34) (0.2 g, yield 58%), as an oil; MS (m/z): 238 (M^+ +1); PMR ($CDCl_3$+DMSO-d_6): 8.4 (s, 1H, H-6), 8.1 (s, 1H, H-3), 5.9 (s, 2H, H-1'), 4.0-4.5 (m, 1H, H-4'), 3.6-3.8 (d, 4H, H-3', 5'), 5.0 (bs, 2H, N-H); (Found: C, 45.4; H, 5.4; N, 35.3 $C_9H_{14}N_6O_2$ requires C, 45.6; H, 5.8; N, 35.4%).

1-[Benzoyloxyethoxy]methyl-4-methylthio pyrazolo[3,4-d]pyrimidine (36)

To a stirring mixture of 19 (3.0 g, 18 mmol), Et_3N (20 ml) and dry DMF (50 ml) was added dropwise a solution of benzoyloxyethoxy methyl chloride (6.0 g, 28 mmol) [prepared by passing dry HCl gas into paraformaldehyde and 1-benzoyloxy-2-hydroxy ethane mixture in dry CH_2CH_2 at 0^o for 2 hr] and stirring continued for 15 hr. The solvent and excess of reagent were removed at reduced pressure. The residue was extracted with $CHCl_3$, washed with H_2O, dried (Na_2SO_4) and concentrated in vacuo. The product thus obtained was chromato-

graphed over SiO_2 column. Elution of the column with $CHCl_3$ gave (36) as an oil (2.2 g, yield 60%); MS (m/z): 344 (M^+); PMR($CDCl_3$): 8.65 (s, 1H, H-6), 7.7-8.1 (s, 2H, Ar-H), 7.1-7.5 (s, 3H, Ar-H), 5.85 (s, 2H, H-1'), 4.1-4.4 (m, 2H, H-3'), 3.6-3.9 (m, 2H, H-4'), 2.6 (s, 3H, $-SCH_2$); (Found: C, 55.8; H, 4.7; N, 16.3 $C_{16}H_{16}N_6O_3S$ requires C, 55.7; H, 4.7; N, 16.4%).

1-[Hydroxyethoxy]methyl-4-methylthio pyrazolo[3,4-d] pyrimidine (37)

A mixture of 36 (2.0 g, 6 mmol) and methanolic NH_3 (45 ml, MeOH at 0^o saturated with NH_3) was kept at ambient temperature for 24 hr. The solvent and excess of reagent from the resulting mixture were removed and the residue was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (96:4, v/v) afforded (37) (1.1 g, yield 55%); m.p.94^o; MS (m/z): 240 (M^+); IR(KBr): 3336 (O-H); PMR($CDCl_3$+DMSO-d_6): 8.8 (s, 1H, H-6); 8.5 (s, 1H, H-3), 5.8 (s, 2H, H-1'), 3.6 (bs, 4H, H-3', 4'), 2.6 (s, 3H, $-SCH_3$); (Found: C, 45.0; H, 5.0; N, 23.3 $C_9H_{12}N_4O_2S$ requires C, 45.1; H, 5.2; N, 23.3%).

1-[Hydroxyethoxy]methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (38)

Method-1

Compound 37 (0.5 g, 2 mmol) in dioxane (60 ml)

was refluxed with aqueous KOH (20%, 15 ml) for 12 hr. The resulting ixture was cooled, neutralized with acetic and the solvent removed at reduced pressure. The residue was extracted with $CHCl_3$, washed with H_2O, dried (Na_2SO_4) and concentrated. The crude product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (90:10, v/v) gave (38) (0.2 g, yield 30%), crystallized in EtOH; m.p.138-40°; UV (MeOH): 250.6, 207.8; (NaOH): 221.2, 215.2; (HCl): 250.8, 212.6; MS (m/z): 210 (M^+); PMR($CDCl_3$+DMSO-d_6: 7.95 (s, 1H, H-6), 7.9 (s, 1H, H-3), 5.7 (s, 2H, H-1'), 3.5 (bs, 4H, H-3', 4'); (Found: C, 45.7; H, 4.8; N, 26.6 $C_8H_{10}N_4O_3$ requires C, 45.8; H, 4.9; N, 26.6%).

Method-2

A mixture of 39 (0.8 g, 2.5 mmol) and methanolic ammonia (25 ml, methanol at 0° saturated with NH_3) was kept for 24 hr at ambient temperature. The solvent and excess of NH_3 were removed at reduced pressure. The crude product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (92:8, v/v) gave (38) (0.5 g, yield 60%), m.p.138-40°.

1-[Benzoyloxy ethoxy]methyl-4(5H)-oxo-pyrazolo[3,4-d]pyrimidine (39)

The compound 24 (3.0 g, 20 mmol) in C_6H_6 (40 ml)

was refluxed with 1-benzoyloxy-2-chloromethoxy ethane (35) (6.0 g, 28 mmol) for 16 hr. The resulting mixture was cooled, filtered. The solvent from the filtrate was removed. The residue extracted with $CHCl_3$, washed with $NaHCO_3$, H_2O, dried (Na_2SO_4) and concentrated. The product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (98:2, v/v) gave (39) (1.7 g, yield 58%); m.p.118-19° (EtOH); MS (m/z): 314 (M^+); UV: 1710 (C=O); PMR($CDCl_3$): 8.25 and 8.1 (each s, 2H, H-6, H-3), 8.0-7.8 (m, 2H, Ar-H adjacent to C=O), 7.45-7.25 (m, 3H, Ar-H), 5.5 (s, 2H, H-1'), 4.5-4.3 (m, 2H, H-3'), 4.1-3.8 (m, 2H, H-4'); (Found: C, 57.3; H, 4.8; N, 17.9 $C_{15}H_{14}N_4O_4$ requires C, 57.4; H, 4.5; N, 17.7%).

1-[Hydroxyethoxy]methyl-4-amino pyrazolo[3,4-d]pyrimidine (40)

A mixture of 37 (0.5 g, 21 mmol) and methanolic NH_3 (20 ml, MeOH at 0° saturated with NH_3) was heated in a steel bomb at 110° for 14 hr. The solvent and excess of NH_3 from the resulting mixture were removed at reduced pressure. The product thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (90:10, v/v) gave (40) (0.3 g, yield 50%);

m.p.153-154^{o} (EtOH); MS (m/z): 209 (M^{+}); PMR($CDCl_3$+ DMSO-d_6): 8.2 (s, 1H, H-6), 8.1 (s, 1H, H-3), 5.7 (s, 2H, H-1'), 3.6 (s, 4H, H-3', 4'); (Found: C, 45.9; H, 5.3; N, 33.5 $C_8H_{11}N_5O_2$ requires C, 45.8; H, 5.3; N, 33.7%).

2.5 BIOLOGICAL ACTIVITY

Antileishmanial Activity

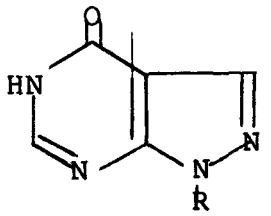
Antileishmanial activity (in vivo) of the nucleosides against amastigotes of Leishmania donovani was determined in hamsters infected with Dd-8 strain according to the procdure described earlier . Allopurinol (17) was used as a standard.drug.

The activity of the compounds (Series A) is given in Table 2. 1,5-Dihydro-4H-pyrazolo[3,4-d]pyrimidine-4-one (allopurinol)(17) exhibited 88% inhibition at 25 mg/kg dose on the 7th day. Substitution of (2-hydroxyethoxy)methyl function which represented (1'-C_4'-C_5') chain of ribose at N_1 of heterocyclic moiety asin (38) rendered the compound inactive. Introduction of [2-hydroxy-1-(hydroxymethyl)ethoxy] function which represented C_1'-(3'-C_4'-C_5') of ribose at N_1 as in (23) considerably increased the activity (75%). The activity was reduced drastically when the hydroxy function at 2 of - [2-hydroxy-1-(hydroxymethyl)ethoxy] group was replaced by an amino function as in (30). The data thus suggested that not only the nature and chain length of glucone moiety at N-1 is critical for antileishmanial activity of the alicyclic nucleosides (Series A) but also the nature of the functional groups present in it.

The antileishmanial activity of nucleosides (Series B) is recorded in Table 2. The activity of the nucleoside (40) was considerably decreased when (2-hydroxyethoxy)methyl function was introduced at N_1 of heterocyclic moiety. However when the hydroxy group in the nucleoside (40) was protected with benzoyloxy function, the corresponding nucleoside (39) exhibited a high order of activity. The compound (26) became inactive when the hydroxy function at C_2 of - [2-hydroxy-1-(hydroxymethyl)ethoxy] moiety was replaced by an amino function. The antileishmanial activity in the Series B type of alicyclic nucleosides, thus, revealed that the nature and chain length and also the nature of the functional group are critical for the activity. Further the high order of activity of the blocked nucleoside (39) indirectly suggested that the compound is probably an inhibitor of some important enzyme involved in the purine salvage process of the parasites.

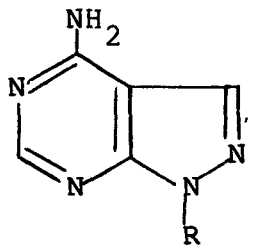
Table 2 Antileishmanial Activity (in vivo) of the nucleosides (Series A and B) at 25 mg/kg on 7th day, against Amastigotes of Leishmania donovani in hamster.

Series A



Compd. No.	R	Inhibition (%)
17	H	88
38	$-CH_2-O-CH_2-CH_2-OH$	0
39	$-CH_2-O-CH_2-CH_2-O-CO-Ph$	82
23	$-CH_2-O-CH(CH_2OH)-CH_2OH$	75
30	$-CH_2-O-CH(CH_2-NH_2)-CH_2OH$	15

Series B



40	$-CH_2-O-CH_2-CH_2-OH$	25
26	$-CH_2-O-CH(CH_2OH)-CH_2OH$	0

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